PRACTICAL METHODS IN MICROSCOPY

CLARK

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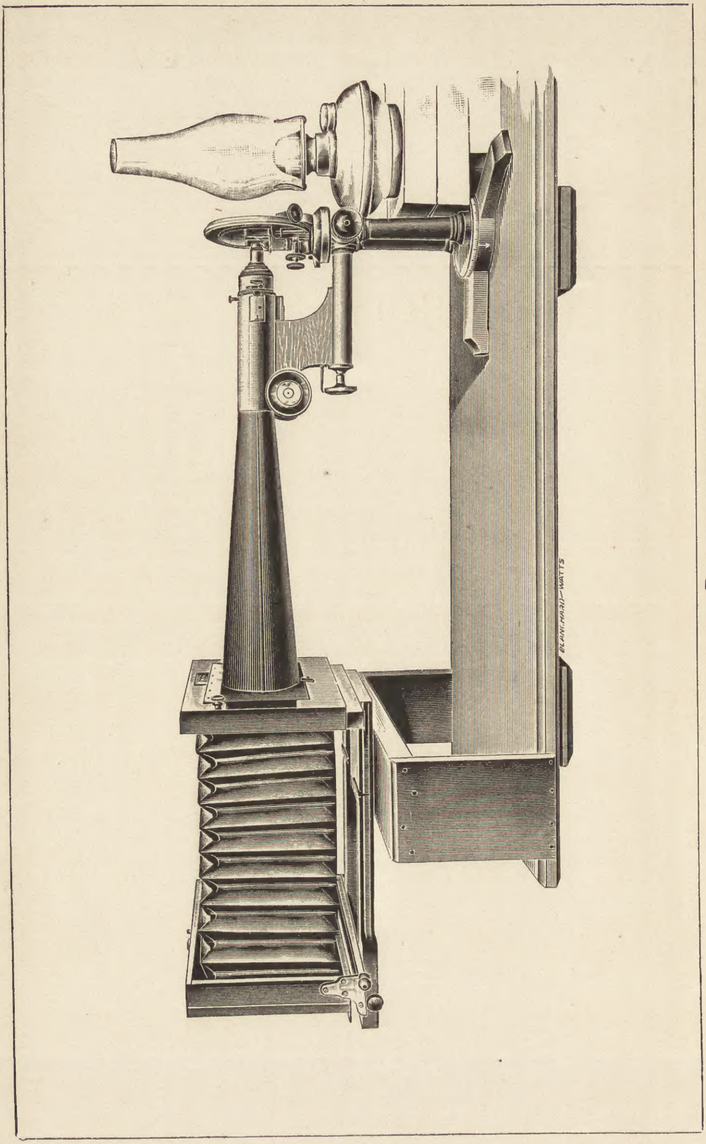

APPARATUS FOR PHOTO-MICROGRAPHY.

FRONTISPIECE.

PRACTICAL METHODS

IN

MICROSCOPY

BY

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PREFACE.

THERE are many excellent books on the microscope, and many on special subjects requiring the use of the microscope as a working tool for investigation. In the most of these books, however, the descriptions of methods are so interwoven with other matters that the inexperienced student is confused and, in many cases, is unable to separate the essential from the non-essential. Too much is assumed to be known, or is left to be filled in by an instructor. None of these books gives to the private worker, in simple and concise language, detailed directions for the many processes that he must learn in order to make practical use of the microscope.

A large amount of apparently unproductive work must be done before the microscope can be advantageously used as a working tool. It is of the highest importance that this work be done at as early a stage as possible, and without waste of time. This book is professedly for beginners and private workers, and is designed to afford the means of acquiring this necessary training. The methods here described are practical methods which have stood the test of general use. They are the methods employed by scientific men the world over. It would not be possible, in most cases, to give credit to the originators of the processes, as the processes have been the result of slow growth, and have been so modified by different workers that they have become common property. Credit can, therefore, be given only in this general way to the books and workers that have furnished the material which has been drawn upon.

These pages are the outgrowth of the author's experience in the use of the microscope in the various branches of scientific study pursued in the secondary schools. They are the result of many hours of pleasant work, and of many hours of laborious reading in the search of what, in many cases, could not be found in the books consulted.

Only so much of the mechanical construction of the microscope and of the theory of light is given as seems absolutely essential to an intelligent understanding of the instrument; other books treat exhaustively of these subjects. The theory of polarized light has been somewhat fully considered, in part because of its important applications and great interest, and in part because this subject is so briefly and so vaguely treated in most books on the microscope. The subject is deep, and it is not easy to treat it in a popular way. An attempt has been made, while adhering to scientific accuracy, to explain in simple language the principal phenomena of polarized light so far as they have practical application in the use of the microscope.

The author wishes to express his sincere thanks to the following gentlemen who have carefully read the proof and offered many valuable suggestions: Professor Leslie A. Lee, Bowdoin College; Dr. S. G. Shanks, Albany, N.Y.; Principal Clarence E. Kelley, High School, Haverhill, Mass.; Dr. S. G. Bonney, Denver, Col.; Mr. George L. Chandler, High School, Newton, Mass.; Rev. J. D. King, Ph.D., Cottage City, Mass.; Dr. W. H. Sylvester, Natick, Mass.

Acknowledgment is also made to the Bausch and Lomb Optical Co., Rochester, N.Y.; Queen & Co., Philadelphia; and Williams, Brown & Earle, Philadelphia, for the use of electrotypes illustrating microscopes and accessory apparatus.

C. H. C.

KINGSTON, N.H., December, 1893.

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CHAPTER I.

LIGHT AND THE MICROSCOPE.

The Undulatory Theory of Light. — The Undulatory Theory of Light assumes that all space is filled with a very rare medium called the ether, which permeates every substance in the universe. The Molecular Theory of Matter assumes that every body of matter is made up of exceedingly minute particles called molecules, which are in a state of constant vibration, moving back and forth in all directions. If a body is heated, the motion of the molecules increases in rapidity as the temperature rises. When this vibratory motion has attained a certain rapidity, the body becomes luminous. This results from the fact that the motion, originating in the molecules of the body and communicated by these molecules to the particles of ether which permeate the body or lie adjacent to it, produces wave motions in the ether, which the ether transmits to the eye. It is known from experimental demonstration that, as the temperature of a body is raised, the wave motions become more rapid, and that the wave lengths in the ether are shortened. When the waves are of lengths within certain fixed limits, which have been determined by experiment, they affect the fibres of the optic nerve

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spread out to form the retina of the eye, and produce the sensation of light. Light is therefore defined, in a subjective sense, as a sensation produced on the retina of the eye by ether waves of certain lengths; in an objective sense, it is a form of energy causing wave movements in the ether.

It is believed that the particles of ether producing these movements vibrate in all directions transversely to the direction in which the light travels, and that the vibrations are sometimes in straight lines, sometimes in ellipses or circles, the particular kind of vibration depending upon the particular vibration of the molecules of the source of the light, or upon the influence of the molecular structure of the bodies through which the light is transmitted on the original wave movement.

Any body heated to such a degree that its molecules cause waves in the ether of certain fixed lengths becomes a source of light. Such bodies are said to be incandescent; the sun and stars are examples. Their heat, which depends on the rate of vibration of the molecules, was caused, when these bodies were formed, by the change of the kinetic energy of the molecules of the masses which clashed together into heat energy, and is maintained by some unknown means. It would be out of place here to discuss the different theories which have been proposed to explain how the heat of these bodies is kept up. In the case of ordinary artificial lights, as a gas light, a lamp flame, or an electric light, molecules of carbon made incandescent by chemical or electrical action are the source of the vibratory motions which are transmitted to the eye by the ether.

If a beam of light is allowed to enter a darkened room through a small aperture, its path through the room is

made visible by the light reflected from the particles of dust in the atmosphere. The path is seen to be straight. The path of light in any homogeneous medium is straight, and its velocity uniform. If, however, the beam of light is made to pass from one medium to another of different density in any other direction than at a right angle to the surface bounding the two media, its path will be bent at the bounding surface, and its velocity diminished or increased according as the second medium is more or less dense than the first medium.

Refraction of Light. — If we place a triangular prism of any transparent substance in the path of a beam of light entering a darkened room, it is easy to see that the beam

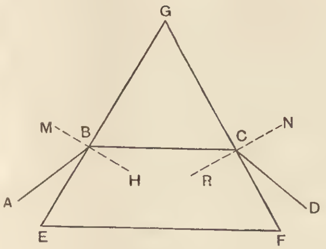


Fig. 1.

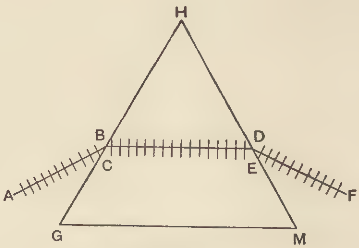


Fig. 2.

is bent at the surface of the prism both on entering and on leaving it, unless the light strikes the surface of the prism at right angles. Figure 1 shows the path of such a beam of light. The ray of light AB strikes the surface EG of a glass prism at an oblique angle. It is bent or refracted at the surface EG towards the base of the prism, passes in a straight line through the glass to the surface GF, and is refracted again towards the base of the prism.

Figure 2 shows Professor Tyndall's way of making clear why the ray is bent. As a wave front approaches the

surface GH of the prism, one end C of the wave reaches the denser medium while the end B is still in the rarer medium. In the denser medium the rate of motion is less than in the rarer medium. The end C moves at a slower rate than the end B until B reaches the glass. A change of direction is the necessary result. When the wave front reaches the surface HM, the end D of the wave reaches the rarer medium while the end E is still in the denser medium. The end D moves more rapidly than the end E, until E reaches the air. Another change of direction results at this surface. It is clear that if the ray falls perpendicularly on either surface, it will not be bent, as both ends of the wave strike the surface at the same instant. It is also easy to see that the more obliquely the ray strikes the surface, the greater will be the refraction. These theoretical facts are easily verified by experiment.

Returning to Fig. 1, the ray, in passing from the air into the glass, is refracted towards the line BH erected perpendicular to the surface bounding the two media at the point B, the point at which the ray enters the prism. The angle ABM is called the angle of incidence for the ray; the angle HBC, the angle of refraction. In passing from the glass into the air, the ray is refracted from the perpendicular CN. In general, a ray of light in passing from a rarer to a denser medium is refracted towards the perpendicular erected on the boundary surface of the two media at the point of incidence of the ray; and in passing from a denser to a rarer medium, the ray is refracted away from the perpendicular. The greater the difference in density of the two media, the greater the amount of the refraction.

Dispersion of Light. — It is also to be noted that a ray of white light passing through a prism is dispersed or divided

into its primary or rainbow colors. This results from the fact that the waves of white light are compound, each wave being the result of the combination of several waves of different lengths. These different waves are differently retarded by the denser medium, the shortest waves, or those which produce the sensation of violet color, being most retarded and as a consequence most refracted; the longest, or those producing the sensation of red, being least retarded and consequently least refracted. The light, then, in coming from the prism exhibits all the colors of the rainbow.

The dispersive powers of different substances are very different. Flint glass has nearly twice the dispersive power of crown glass. This suggested to Dollond, a London optician, a means of effecting refraction without dispersion. He combined a crown glass prism *ABC* with a flint glass prism *CDB*, Fig. 3, with the edges turned in opposite directions. By a careful adjustment of the size of the angle *CBD* to the angle *ACB*, the rays which are separated in the prism *ABC* are caused to combine in the prism *BCD*. In this way refracted light which is nearly but not quite achromatic is obtained. This principle has an important application in the construction of achromatic objectives.

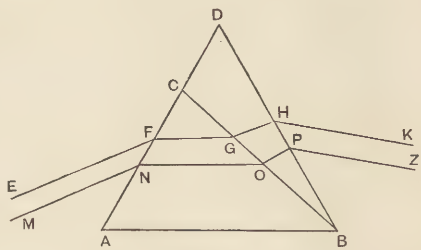


Fig. 3.

Index of Refraction. — Let *AB*, Fig. 4, represent the boundary surface between two media, *e.g.* glass below, air above. *CO* is a ray of light entering the glass at *O*. It

is refracted towards the perpendicular *OR* and passes along the line *OD*. With *O* as a centre and with any radius describe a circle, as *AEBF*. From the point *M* where the incident ray cuts the circumference draw *MH* perpendicular to *EF*. The angle *COE* is the angle of incidence, and *MH* is the trigonometrical sine of the angle of incidence for the ray *CO*. From the point *P* where the refracted ray cuts the circumference, draw *PR* perpendicular to *EF*. *POF* is the angle of refraction, and *PR* the sine of the angle of refraction for the ray *CO*.

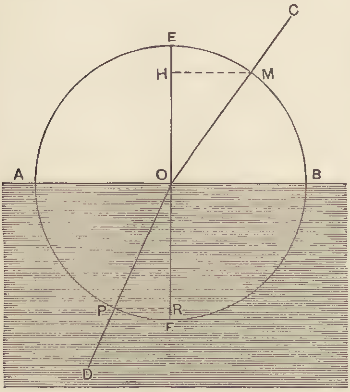


Fig. 4.

Notwithstanding that it is true that the refraction of the ray increases as the angle of incidence increases, it has been found by experimental demonstration that, as long as the two media remain the same, the ratio of the sine of the angle of incidence to the sine of the angle of refraction is constant. This ratio $\frac{MH}{PR}$ is called the Index of Refraction. It is usually expressed decimally. If the ray is passing from a rarer to a denser medium, the index of refraction is greater than unity; if the ray is passing from a denser to a rarer medium, the index of refraction is less than unity.

The index of refraction for a ray of light passing from a vacuum into any medium is called the absolute index of refraction for that medium. The absolute indices of vari-

ous substances have been experimentally determined. The following table, compiled from several authentic sources, gives the absolute indices of refraction for some of the media that are of importance to the microscopist: —

Absolute Indices of Refraction.

Air	1.000294	Plate glass (glass slips and cover-glasses) .	1.53
Pure water	1.333	Homogeneous immersion fluid	1.51
Canada balsam . . .	1.54	Iceland spar, ordinary ray	1.654
Oil of cloves	1.53	Iceland spar, extraordinary ray . . .	1.654–1.483
Oil of turpentine . .	1.478		
Oil of cedar	1.51		
Crown glass	1.51		
Flint glass	1.62		

The relative index of refraction for a ray of light passing from one medium to another is found by dividing the absolute index of the medium into which it is passing by the absolute index of the medium from which the ray is coming. If the ray is passing from a rarer to a denser medium, this quotient is greater than unity, and the ray is refracted toward a line erected at the point of incidence perpendicular to the surface bounding the two media. If the ray is passing from a denser to a rarer medium, the quotient is less than unity, and the ray is refracted away from the perpendicular. As the index of refraction shows at a glance which way the ray is bent, and whether it is bent much or little, it is a very important datum. An understanding of the index of refraction is of great practical importance in studying immersion objectives and the effects of the cover-glass.

Total Reflection. — Intimately connected with refraction is total reflection, which, because of its practical bearing in the construction of the polarizing apparatus used on the

microscope, requires attention here. Place the eye on a level with a gas or lamp flame and raise a glass of water steadily above this level between the eye and the flame. When the water is held in a particular position, the flame is seen reflected as from a mirror from the under part of the upper surface of the water. Fig. 5 shows the reason. Let *AB* be the surface of the water. The ray of light *EO* in passing from the water into air is refracted away from the perpendicular *CO* along the line *OF*. The ray *GO* is refracted along *OH*, the angles of refraction increasing more rapidly than the angles of incidence. If we continue to take the successive rays, a ray will be found, as *B′O*, which strikes the surface *AB* at such an angle that the angle of refraction *COB* is a right angle. The refracted ray just grazes the surface of the water, passing along the line *OB*. All rays between *B′O* and *AO* cannot pass out of the water, but are totally reflected from the surface. The angle of incidence *B′OD* is called the critical or limiting angle. When the light strikes the surface of a transparent medium at an angle greater than the critical angle, this surface is a perfect mirror. The critical angle for water is about 48° 30′. It is different for different substances. For the diamond it is 23° 41′. The brilliancy of the diamond and other gems is due to repeated total reflections of the light within the gem.

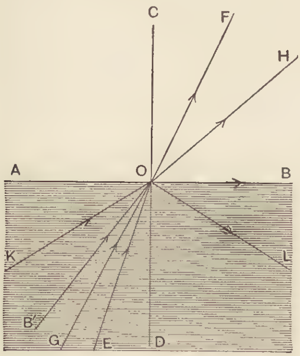


FIG. 5.

The Double-Convex Lens.—Fig. 6 represents two prisms placed base to base and the paths of rays of light from the

point *F*. It is seen that the rays which diverge from the point *F* converge, after passing through the prism, to

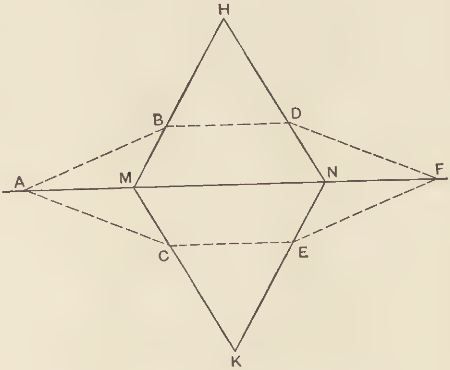


FIG. 6.

the point *A*. This arrangement of prisms represents very well the principle of the double-convex lens.

The surfaces of a double-convex lens may be considered as made up of an infinite number of small plane surfaces. Each ray of light coming from a given point, except the

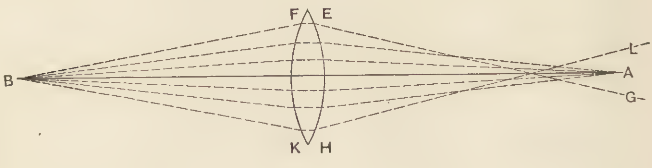


FIG. 7.

one passing along the optic axis *BA*, Fig. 7, is refracted towards the optic axis both on entering and on leaving the lens. The result is that rays coming from the point *B* are refracted to the point *A*.

Spherical Aberration. — The surfaces of convex lenses are usually portions of spheres. As the incident rays approach the edge of the lens, the angles of incidence increase very rapidly, and the refraction of the rays is, of course, correspondingly increased. The result is that those rays which pass through the lens near its edge, as *BF* and *BK*, Fig. 7, are brought to a focus sooner than the rays which pass through the central portion of the lens. There results a blurring of the image. This defect of convex lenses is remedied by interposing a diaphragm, a piece of opaque substance with a hole through it of a diameter somewhat smaller than the diameter of the lens, to cut off these objectionable rays. Diaphragms are used in all the optical parts of the microscope, as the eye-piece, objective, condenser.

Dispersion in Lenses. — Another defect in convex lenses of homogeneous material is dispersion of the light, whereby color effects due to the separation of white light into its primary colors are produced. From the shape of the lens this separation is not so great as in the case of the prism, yet there is enough to give circles of prismatic colors around the edge of the field of view. This defect is remedied by combining, on the principle of Dollond, convex and concave lenses of crown and flint glass so adjusted that the dispersion of the one is corrected by the opposite dispersion of the other, in a manner similar to that already explained under prisms. The correction can, however, be perfectly made for only two colors at a time, so that lenses entirely free from dispersion cannot be made. The skill of the opticians has produced lenses which would seem to be as free from this defect as human ingenuity can make them. Fig. 8 shows such a compound lens. Ordinarily the convex lens is of crown glass and

the plano-concave lens of flint glass, but for some purposes the order is reversed. These lenses are called achromatic, and are used in the construction of all optical instruments of high grade. Apochromatic objectives are a modern triumph of the opticians in which the correction is practically made for three colors.

FIG. 8.

The Simple Microscope. — Every double-convex lens is a simple microscope. The manner in which it acts is illustrated in Fig. 9. A ray of light coming from the point A on the arrow AB, placed just within the principal focus of the lens, is refracted on entering the lens and again on leaving it, passing in the line HK to the eye. The eye looking along the line KHA' seems to see the point A at A', the position being determined by the intersection of the line KA' and the secondary axis PA'; similarly the point B appears at B'. In like manner we could draw lines to show that all the rays coming from all the points of AB would be so refracted that they would appear to come from points on $A'B'$. We thus see an enlarged image of the arrow AB in the position $A'B'$. It is to be noted that the image is not inverted.

FIG. 9.

A simple microscope consists, then, of one or more convex lenses mounted in such a way as to be convenient to use for the purpose for which the microscope was designed. Any lenses of the convex series — double-convex, plano-

convex, or concavo-convex — may be so employed. The manner of action is precisely the same in all, but is less in amount in the plano-convex and concavo-convex lenses than in the double-convex. Figs. 10, 11, and 12 show different manners of mounting simple microscopes.

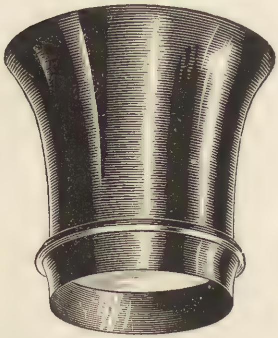


FIG. 10.

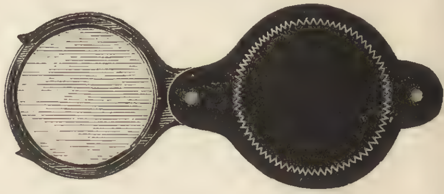


FIG. 11.

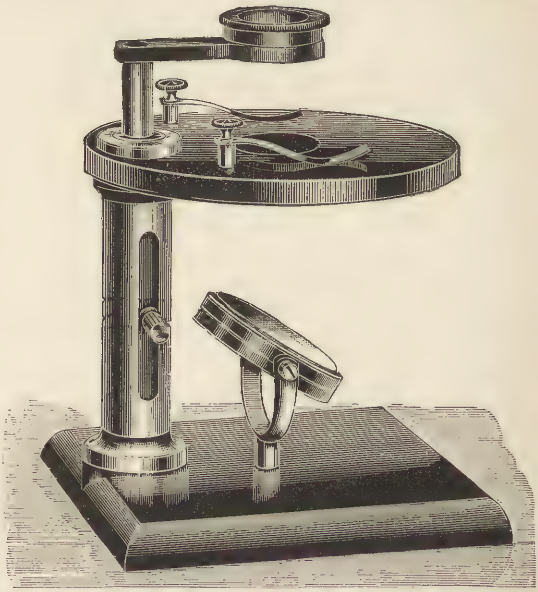


FIG. 12.

The Compound Microscope. — The simplest form of compound microscope consists of two convex lenses: one small and placed near the object; the other larger and placed in such a position that the image formed by the small lens is in its focus. The small lens is called an

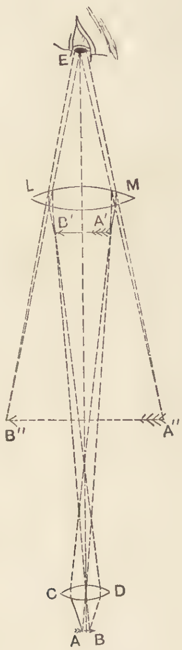


FIG. 13.

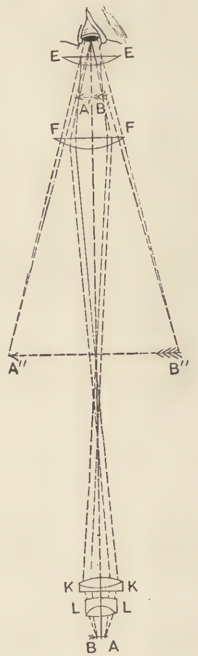


FIG. 14.

objective from the fact that it is placed near the object; the larger lens, an eye-piece or ocular, from the fact that the eye is placed near it to make observation. Fig. 13 shows how the objective forms a real, inverted image of the object AB at $A'B'$. If a screen of oiled paper or delicately ground glass is placed here, the inverted image

of the object will be seen upon it. The eye-piece acts as a simple microscope to magnify this image.

In practice, an objective consists of two, three, or four achromatic convex lenses placed near together. The lens nearest the object is called the front lens or front combination; the one farthest from the object is called the back combination. These different lenses act together to magnify the object. Two or more lenses of slight curvature are used rather than one of considerable curvature, as it is found that spherical aberration and dispersion are thus reduced to a minimum.

The eye-piece sometimes consists of a single convex lens. It is then known as a positive eye-piece and is really a simple microscope, as *LM* in Fig. 13. More commonly a Huyghenian eye-piece is used. This consists of two convex lenses. The one nearest the eye is called the eye-lens; the other, the field-lens. The field-lens acts precisely as one of the lenses of an objective, and is regarded by many as constituting a part of the objective. The rays of light from the object, after passing through the objective, pass through the field-lens of the eye-piece before coming to a focus. Refracted by the field-lens they form the inverted image of the object between the field-lens and the eye-lens, as $A'B'$, Fig. 14. If the eye-lens is removed and a piece of oiled paper lowered into the eye-piece, an image of the object will be seen on it when it is carefully held transversely in the

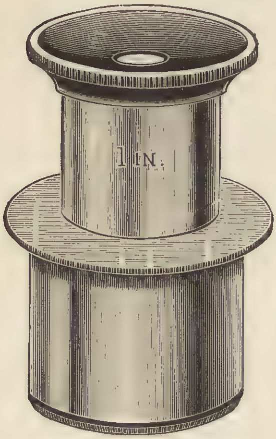


FIG. 15.

tube at the proper distance from the field-lens. The diaphragm of the eye-piece is in the plane where the image is formed. When an eye-piece micrometer is used, it is placed in this position. The eye-lens acts as a simple microscope and magnifies this image. It is thus seen that with the compound microscope the image appears inverted whether the positive eye-piece or the negative Huyghenian eye-piece is used.

The compound microscope is then an application of convex lenses. The stand with all its various parts is only a convenient means to render the use of the lenses easy and bring out their full power. In Fig. 16, *A* is the objective, *B* the eye-piece, *C* the tube or body, *D* the draw-tube, *E* the nose-piece which adapts the objective to the body, *F* the stage, *G* the sub-stage which receives various accessories, *H* the mirror, *K* the mirror-bar, *J* the base, *L* the flexible pillar, *O* the arm, *M* the coarse adjustment by rack and pinion, *N* the fine adjustment. On the stage is seen a microscope slide, three inches by one in dimensions, held in position by spring clips.

Selection of a Microscope. — Much has been written in regard to the selection of a microscope, but to very little purpose. It is impossible for one person to give another very definite practical advice on such a matter. Tastes, purses, and the object for which the instrument is to be used differ. A few facts should, however, be borne in mind. Very cheap microscopes are useless for practical purposes. It is poor economy for a beginner to suppose that a cheap stand is good enough while learning to use the microscope. It is good economy, although the first outlay will be somewhat greater, to purchase a stand of thorough workmanship, of sufficient steadiness to bear any objective, and of such construction as to receive any of

the sub-stage accessories. Such a stand admits of the highest character of work and of unlimited development in microscopical skill on the part of the possessor. It must, of course, be used with especial care by the beginner, but the exercise of this care is the very best means to educate one to a careful and painstaking system of working, the secret of success in microscopical manipulation. The prospective purchaser will, of course, consult his microscopical friends and learn what he can about their instruments and receive the benefit of their experience. He will also obtain the catalogues of several of the most reputable makers of microscopes, compare the instruments and prices, and will purchase only of makers of established reputation, who place their names on their apparatus as a guarantee that it is what it is represented to be.

For a beginning, the stand with two eye-pieces, *A* and *C* (the latter fitted with an eye-piece micrometer), and two objectives, a three-fourths or two-thirds inch and a one-fifth or one-sixth inch, is ample equipment. As knowledge of the instrument and experience in its use are acquired, other parts may be added. An error that is commonly made in first purchases is to obtain a multiplicity of accessories that are afterwards found to be useless. It is much the wiser course to purchase accessories as they become actual necessities.

Care of the Microscope. — The microscope should be kept scrupulously clean in all its parts. The metal portions may be wiped along the grain of the finish with a piece of old, soft linen. The draw-tube should be thus wiped occasionally. No alcohol or other fluid which dissolves resinous substances should be allowed to come in contact with the metal, or the lacquer finish will be dissolved.

Dust may be removed from the lenses with a piece of

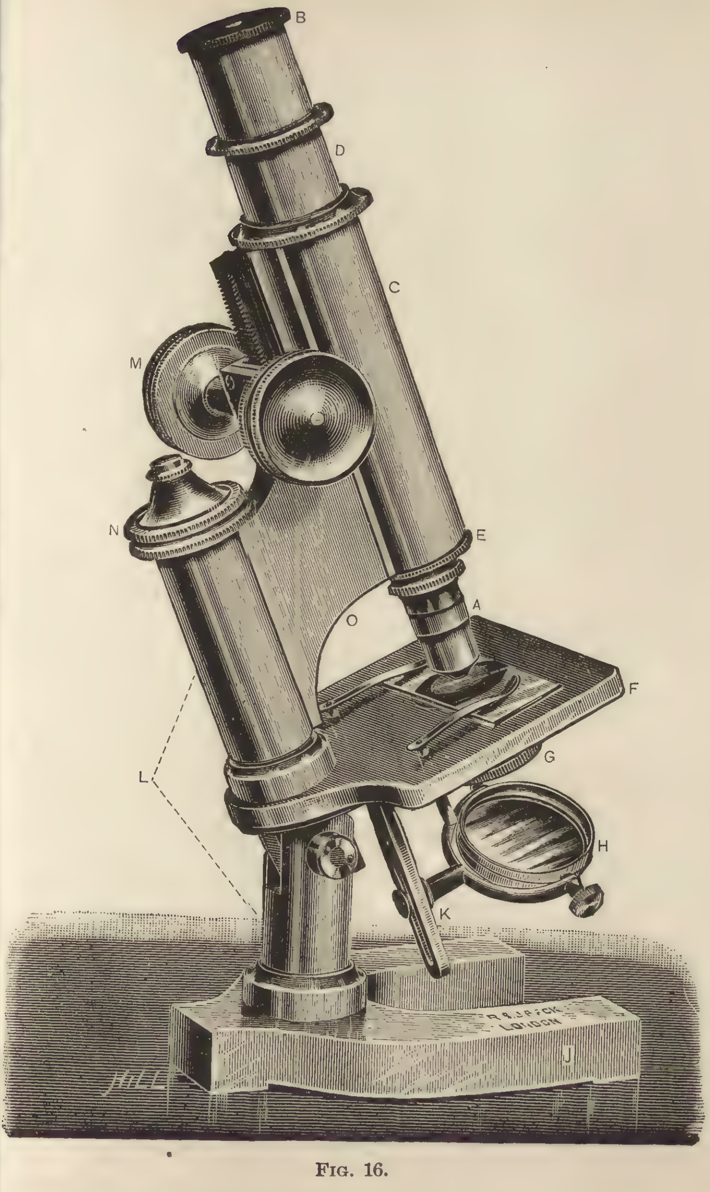


FIG. 16.

old, soft linen or a camel's-hair brush. One or two folds of the linen over the end of a stick of pine wood, previously chewed until it is quite soft, serves well to reach the smaller lenses. Professor Gage of Cornell University recommends very highly for cleaning lenses the bibulous Japanese filter paper much used by dentists. Do not touch the lenses with the bare fingers. After wiping, breathe upon the lens. If it is optically clean, the moisture will disappear almost instantaneously. If it is not clean, the moisture will persist.

To remove the objective from the stand, lightly grasp the lower part, without touching the glass itself, with the thumb and forefinger of one hand to guard against dropping the objective. Place the thumb and forefinger of the other hand on the milled rim at the upper part of the objective and unscrew it. In replacing the objective on the stand, use the same precautions. The lenses of the objective should not be separated unless one has had considerable experience with optical apparatus, and in the case of the higher power objectives it is perhaps best to send them to the makers of the instrument if they need to be taken apart for any reason. It will seldom occur that this is necessary.

In the course of use the front lens of the objective is liable accidentally to come in contact with the reagent used in the study of the specimen. It should be wiped immediately. If it has touched Canada balsam or other resinous substances, it may be wiped with a piece of linen moistened with alcohol, taking great care that the alcohol does not come in contact with the brass mounting of the objective. After using the alcohol, wipe immediately with dry, soft linen.

Specks in the field of view are most commonly due to

dust on the field-lens of the eye-piece. Dust is liable to collect on both the field-lens and the eye-lens. Either lens may be unscrewed from its mounting and wiped, with the precautions already mentioned, which apply to the cleaning of any of the optical parts of the instrument.

To determine when the dust has been entirely removed, place the eye-piece in the tube, focus sharply on a well-lighted field, and then slowly revolve the eye-piece. If there are any particles of dust on either of its lenses, they will revolve, while dust specks on the slide, mirror, or objective remain stationary. To ascertain if the dust is on the slide or mirror, move first one, then the other. The dust will move with the slide or mirror, if there is any on either. If, after these manipulations of the eye-piece, slide, and mirror, there are still dark patches which have not moved, there is dirt on the objective.

The Glass Slip. — Objects to be examined under the microscope are placed upon glass slips three inches long and one inch wide. These should be purchased ready for use. Only those with ground edges should be used, and it is economy in satisfaction, if not in money, to buy a good quality. Recent improvements in the processes of manufacture, and the increased demand arising from the great advance in the popular use of the microscope in the past few years, have caused large reductions in the prices of the slips, so that the purchase of the best is now no great burden. Slips with unground edges are very liable to leave serious scratches on the stage of the microscope. Slides made from such slips are unsightly and lessen the ambition to produce perfect work. It is a waste of time, which can better be given to something else, to attempt to grind one's own slips.

The Cover-Glass. — If the object is to be examined in any fluid medium, it *must* be covered with a piece of thin glass, known as the cover-glass. This is used in part to protect the objective, in part to flatten the object, but more particularly to prevent the refraction of the light which would occur at the curved surface of an uncovered drop of fluid. This refraction of the light would cause a very distorted image of the object to be formed. The curved surface of the drop would act precisely as the curved surface of a lens. The cover-glass itself exerts a very great influence on the light, but this influence is far less objectionable than that of a distorted drop of water.

The thickness of the cover-glass is also a matter of importance. A study of Fig. 17 will make plain how the thickness of the cover-glass affects the clearness of the image. The figure is drawn for a dry mount. Let A represent a point on an object, and EF the cover-glass. A ray of light, AB, from the object, on striking the lower side of the cover-glass, is passing from a medium, air, whose refractive index is 1.000294, into another medium, plate glass, whose refractive index is 1.53. The relative index of refraction is 1.52. The ray is therefore refracted towards the perpendicular MN erected on the lower surface of the cover-glass at the point where the ray strikes. On passing from the cover-glass into air, the ray is passing from a medium whose refractive index is 1.53, into a medium whose refractive index is 1.000294. The relative index of refraction in this case is .65. The ray is

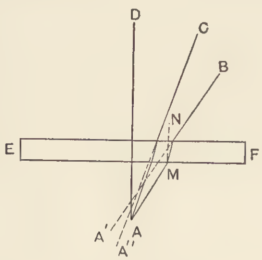


Fig. 17.

now refracted away from the perpendicular, and as the conditions in the second refraction are the exact reverse of the conditions in the first refraction, its course after leaving the upper surface of the cover-glass is parallel with its course before entering the cover-glass. The ray has suffered displacement. $A'B$ shows the direction from which it appears to come, and the point where the line $A'B$ crosses the optic axis of the microscope, DA, shows the position in which A appears to the observer in so far as this particular ray helps to form its image. It is apparent from an inspection of the figure that the greater the thickness of the cover-glass, the greater the displacement of the ray. It is also to be remembered that this displacement is magnified when observed through the microscope. The line $A''C$ shows the apparent direction of the ray AC. Should we, in like manner, construct lines for other rays coming from the point A and trace the directions back, we should see that all are displaced in varying degrees. The result is, of course, the blurring of the image.

This defect of the cover-glass can to a considerable extent be remedied in the construction of the objective. Except in the case of collar adjustment objectives, which will be considered later, it must be done for a particular thickness of cover-glass. It is accomplished by giving the lenses such shapes, and placing them at such distances from one another, that the rays which have been displaced are refracted back, so that they appear to come from their real source, the point A in Fig. 17. Microscope makers correct their non-adjustable objectives for the particular thickness of cover-glass and for the particular length of tube which they have severally adopted. It is unfortunate that the makers have not agreed upon the same thickness

of cover and the same tube length. The tube length adopted by opticians of high reputation varies from 125 to 254 millimetres. The thickness of covers adopted varies from one-tenth to twenty-five hundredths of a millimetre. If an objective corrected for a cover one-tenth of a millimetre thick be used on one twenty-five hundredths of a millimetre thick, the image will be blurred, and *vice versa.* If it is necessary to use cover-glasses that are too thick or too thin, the defect can to a considerable extent be remedied by using a greater length of the draw-tube when the cover is thinner than the standard thickness for which the objective was constructed, and by shortening the draw-tube, if the stand is constructed so as to allow it to be done, when the cover is too thick. In examining permanent mounts, when the thickness of the cover-glass is not known and cannot be measured, the proper length of the draw-tube should be found by trial. Focus sharply with the tube of the length for which the objective was constructed. Then lengthen or shorten it until the clearest image possible is obtained. Such intelligent use of the draw-tube aids greatly in securing a clear image and in bringing out the highest capabilities of the objective. The foregoing considerations show that it is very important to know the thickness of the cover-glass.

Cover-Glass Gauge. — Several devices have been contrived for measuring the thickness of the cover-glass. A very commendable form has recently been placed upon the market at a reasonable price by the Bausch and Lomb Optical Company. It is shown in Fig. 18. The cover-glass is placed in the slot provided for it, and the micrometer screw turned until the end touches the cover-glass. The thickness in fractions of a millimetre or of an inch is then read directly from the drum. The drum also

shows directly the correct tube length to use for any thickness of cover, when objectives of one-fourth, one-fifth, one-eighth, and one-twelfth inch focus, corrected for

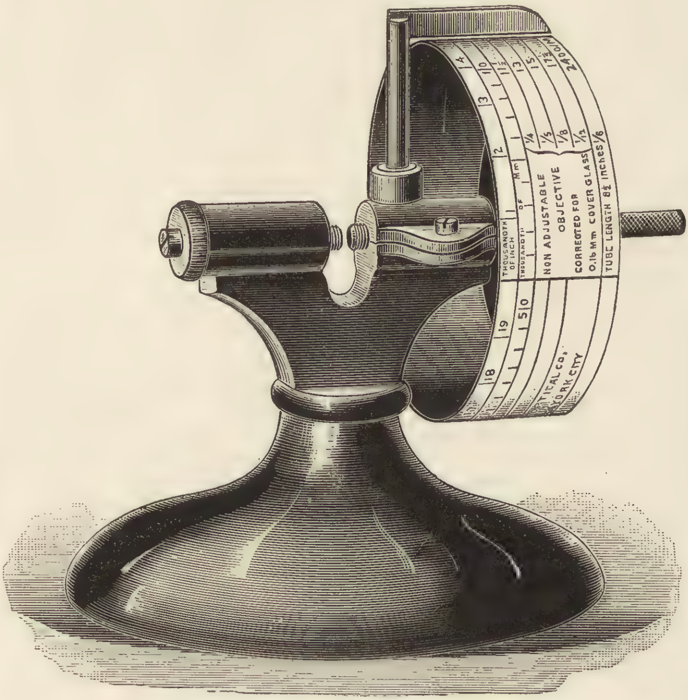


FIG. 18.

cover-glasses .16 of a millimetre thick, and for a tube length of eight and one-half inches, are used. It is a very convenient and useful device.

CHAPTER II.

SOME ACCESSORIES FOR ADVANCED WORK.

Immersion Objectives. — For the more advanced histological, and especially for bacteriological work, high power immersion objectives are required. These may be divided into two classes, those with which water is used as the immersion fluid, and those with which an oil is used, usually cedar oil, or some fluid especially prepared by the opticians, and having a refractive index about the same as the refractive index of the front lens of the objective. This fluid is called homogeneous immersion fluid, as it is homogeneous as respects its refracting and dispersing power with the glass of the front lens. The objective is called a homogeneous immersion objective. Objectives used with water are called water immersion objectives.

In using an immersion objective, a small drop of the fluid is placed on the front of the front lens or on the cover-glass, and the objective carefully lowered until the fluid forms a connection between the objective and the cover-glass. The objective is then focussed by means of the fine adjustment, and, if the objective is adjustable, by the collar adjustment. The principle upon which an immersion lens works and its superiority over a dry objective will be apparent from a study of Fig. 19.

A ray of light starting from the point *A* of the object on the slide, passes from the balsam to the upper surface of the cover-glass with only a slight change of direction at the lower surface of the cover-glass; for, as will be seen

by inspecting the table of Indices of Refraction given in Chapter I., it is passing from a medium, Canada balsam, whose refractive index is 1.54, into another medium, the cover-glass (plate glass), whose index of refraction is 1.53, or from a denser to a slightly rarer medium. The relative index of refraction is .993, which shows that the ray is slightly bent away from the perpendicular.

If now a dry objective is used, the ray, in passing from the upper surface of the cover-glass into the air, is passing from a medium whose index of refraction is 1.53 into a medium whose index of refraction is 1.000294. The rela-

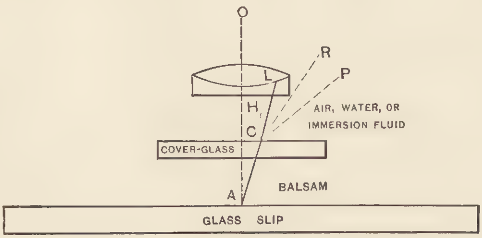


FIG. 19.

tive index of refraction is .653, which shows that the ray is much bent away from the line *CH*, erected at the point where the ray strikes the upper surface of the cover-glass perpendicular to that surface. This perpendicular is, of course, parallel to the optic axis of the microscope. The ray takes some such direction as *CP* and fails to reach and enter the front combination of the objective.

If, instead of the dry objective, a water immersion objective is used, the ray, in passing from the cover-glass into water, is passing from a medium whose refractive index is 1.53 into a medium whose refractive index is 1.333. The relative index of refraction in this case is .871.

This shows that the ray is still refracted away from the perpendicular to a considerable extent, but by no means to so great an extent as when the dry objective was used. The ray takes some such path as *CR*, and also fails to reach and enter the front lens of the objective; but it is evident that many more rays will enter a water immersion objective than can enter a dry objective of the same angular aperture and working distance.

If we use a homogeneous immersion objective, the ray, in passing from the cover-glass into the homogeneous immersion fluid, is passing from a medium whose refractive index is 1.53 into a medium whose refractive index is 1.51. The relative index of refraction is .987. The ray is but slightly refracted away from the perpendicular. It takes some such path as *CL*, reaches and enters the front lens of the objective without refraction, as the refractive index of the front lens (crown glass) is the same as the refractive index of the homogeneous immersion fluid. It is evident that the homogeneous immersion objective receives a much larger number of rays from the point *A* than does a water immersion objective of the same angular aperture and working distance.

The importance of causing the largest possible number of rays from the object to pass through the objective is manifest, when we consider that the perfection of the image depends on the number of rays that help to form it. The gain in light is also of great consequence, for, with high-power objectives having three and four combination-lenses, the absorption of light is considerable.

Adjustable Objectives. — To remedy the evil effects of changes in the thickness of the cover-glass, and to compensate for variations in the refractive index of different cover-glasses, and for changes in the tube length,

which result when different eye-pieces are used, high-power objectives, both dry and immersion, are made with means of adjusting the distance between the front and back combinations of the objective. Theoretically, homogeneous immersion objectives do not require to be made adjustable, and many of them are not so made; but because the conditions for which they are made can seldom be perfectly met, because of the variation in the refractive indices of different cover-glasses, and because the immersion fluid and other factors which influence the working of the objective are liable to vary, the highest grade homogeneous immersion objectives are made adjustable: water immersion objectives are always made adjustable.

An objective is made adjustable by mounting either the back or the front combination so as to be moved up or down by means of a milled collar. This collar is graduated arbitrarily, and when the adjustment is at the medium point, the objective is corrected for the standard thickness of cover-glass, and the standard length of tube used for all objectives by the maker. When the adjustment is at the lowest point, the front and back combinations are separated and adjusted for the thinnest cover-glasses. When the adjustment is at the highest point, the lenses are brought nearest together and adjusted for thick covers. In some objectives the front combination is movable; in others, the back combination. The latter arrangement is the one now in common use, as the objective is much more easily handled.

The manipulation of adjustable objectives calls for the highest skill of the microscopist. Only practice gives proficiency in handling them. General directions can be given, but much must be left to the patient perseverance

of the worker. Some useful hints on their manipulation are given in Chapter IV.

Angular and Numerical Aperture. — Since the perfection of the image depends upon the number of rays which help to form it, the efficiency of an objective depends upon the number of rays it can transmit. Dry objectives were long compared with one another by comparing the angles formed by the most divergent rays which can pass through the objective from a point on the object situated on the optic axis of the microscope. These angles are called angles of aperture. In a similar way, water immersion objectives were compared with one another, and also homogeneous immersion objectives were compared among themselves. There was even confusion, and objectives of one kind were compared with those of another kind by the angular apertures. Even when objectives of the same kind are compared, only rude ideas of the relative efficiencies are obtained. The efficiencies are really to one another as the sines of one-half the angles of aperture.

If, however, we wish to compare dry, water immersion, and homogeneous immersion objectives with one another, we must find some means of comparing the number of rays which can pass through these different kinds of objectives. Professor Abbe of Jena first demonstrated the fact that the relative efficiencies of objectives could be expressed by the numbers obtained by multiplying the sine of one-half the angle of aperture by the index of refraction of the immersion fluid employed. The result is called the numerical aperture. It is calculated by the formula $\text{N.A.} = n \sin u$, where N.A. stands for *numerical aperture*, n for the *index of refraction* of the immersion fluid employed, if the objective is an immersion

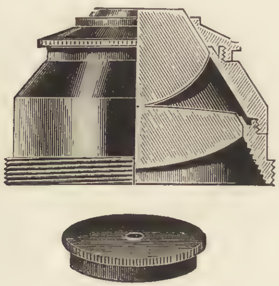
objective, or of air, if the objective is used dry, and sin u, for the *sine of one-half of the angle of aperture* of the objective.

If, for example, we wish to compare three objectives each $\frac{1}{8}$-in. focus, the first, dry, angular aperture 135°; the second, water immersion, angular aperture 170°; the third, homogeneous immersion, angular aperture 138°; they do not compare as the angles 135°, 170°, and 138°, but obtaining the sines of one-half of each of these angles from a table of natural sines, and making the substitutions in the above formula, we have for the dry objective, N. A. $= 1.000294 \times .9239 = .924$; for the water immersion objective, N. A. $= 1.33 \times .9962 = 1.32$; for the homogeneous immersion objective, N. A. $= 1.51 \times .9336 = 1.41$. The efficiency of these three objectives is then as .924, 1.32, and 1.41.

By a similar application of the formula, it may be seen that objectives of 82° homogeneous immersion, 96° water immersion, and 180° dry, are practically of the same efficiency in transmitting the light from the object.

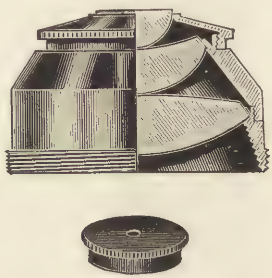
Sub-stage Condenser. — On account of the great absorption of light by high-power objectives, a sub-stage condenser must be used in the more advanced studies with the microscope. The condenser is a lens or system of lenses which converge the light upon the object. A medium-power objective makes a very good condenser when the sub-stage is so arranged that the objective can be attached with its front lens towards the object, and moved up and down until the object on the slide is in its correct focus; for, like any other lens, the sub-stage condenser, whatever its construction, must be focussed, and upon the accuracy with which this is done depends its usefulness.

For fine work, however, the condenser must be of special construction. Figs. 20 and 21 show the construction of different forms of the Abbe condenser as made by the Bausch and Lomb Optical Company. The working distance of these condensers is very short, so they must be placed close up to the slide; and as the thickness of the glass slip is variable, the condenser must be specially focussed for each new slide used. The better classes of sub-stages are provided with rack and pinion to



Optical part of Condenser 1.20 N. A.

FIG. 20.



Optical part of Condenser 1.42 N. A.

FIG. 21.

accomplish this. Centring-screws are also provided in the sub-stage to centre the condenser correctly. In centring, a pin-hole cap, provided for the purpose, is placed over the front lens, and the centring-screws adjusted until the opening is in the centre of the field. In using the condenser, it is very important to see that it is correctly centred and correctly focussed.

These condensers may be converted into immersion condensers by placing a drop of water or of immersion fluid on the front lens, and making contact with the lower side of the slide.

The Camera Lucida. — The camera lucida is an attachment to the eye-piece of the microscope, whereby the image may be seen projected upon the surface of a sheet of paper. One of the simplest forms is shown in Fig. 22. To use this form, the microscope is inclined. The drawing surface must be placed parallel with the tube. The image of the object is then seen without distortion when the eye is placed so that

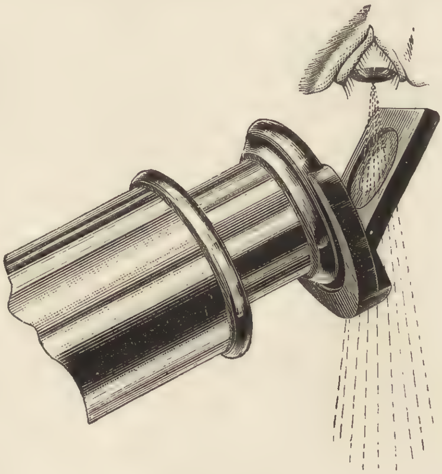


Fig. 22.

the axial ray from it to the drawing surface is perpendicular to the latter. The principal difficulties to be overcome arise from the indistinctness of the pencil point, and from the fact that the image is inverted.

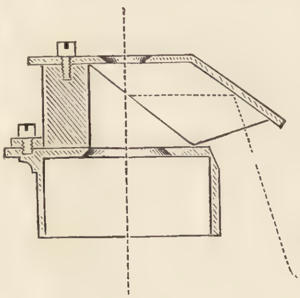


Fig. 23.

Fig. 23 shows the principle upon which the Abbe camera lucida is constructed, and Fig. 24 shows an improved form. These are the best forms of the camera lucida made. They are designed primarily to be used with the microscope in the upright position, but may also be used with the instrument inclined. To render the

pencil point more distinct, a prism made up of a wedge of smoked glass and a counterbalancing wedge of clear glass is provided to be mounted in spring clips. By means of this prism a varying thickness of the smoked glass may be placed in the path of the rays which form the image, until the pencil point is distinctly visible.

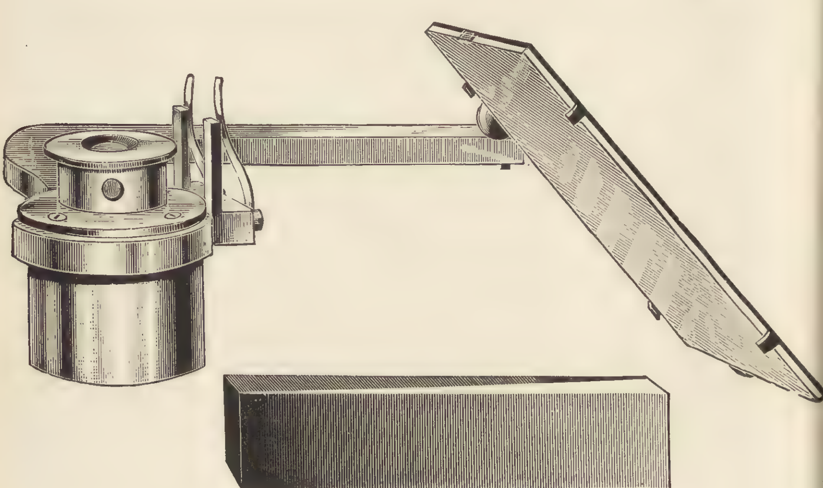


FIG. 24.

The drawing surface should be shaded from all extraneous light.

The educational value of drawing the objects which are being studied cannot be too highly estimated. In no other way can the structure be so easily and clearly fixed in the mind. Points of structure are thus frequently called to the attention which otherwise would be entirely overlooked. The process is slow and tedious, but measured by the knowledge gained the results are all valuable.

Micrometers and Micrometry. — There are two simple ways of measuring the size of an object under the microscope. The first is by means of a stage micrometer. A stage micrometer is simply a scale ruled on glass by a dividing engine. The divisions are usually $\frac{1}{1000}$, $\frac{1}{100}$, and $\frac{1}{10}$ of an inch, or, in the metric system, $\frac{1}{100}$ and $\frac{1}{10}$ of a millimetre. The stage micrometer is placed on the stage, and the object placed above it. The size is read directly. Theoretically this is the simplest way to measure an object, but practically there are almost insurmountable difficulties in the way of accurate work. Unless the object is a perfect circle it cannot be made to coincide with the micrometer in more than one direction, and it is probable that it will not coincide even in that one direction, for neither the micrometer nor the object can be conveniently revolved so that the graduations may coincide with the dimension to be measured. It is, then, usually the case that the measurements made with the stage micrometer are crude and lack much in accuracy.

The second way of measuring is by means of an eye-piece micrometer. By this method the object is not directly measured, but the image of it formed in the eye-piece. The eye-piece micrometer is also a scale ruled on glass. The rulings are generally made $\frac{1}{10}$ of a millimetre from one another. The slip of glass is inserted in the eye-piece just above the diaphragm, and in such a position that the scale is exactly in the plane in which the image of the object is formed. As it is the image of the object and not the object itself that is measured, it becomes necessary to know how much the image has been magnified. This differs with objectives of different focal lengths, and also to some extent even with objectives marked as of the same focal length. It is necessary, then,

to determine this magnification for every objective used. This is done by placing an accurate stage micrometer on the stage, and using it as an object. Observe how many of the divisions of the eye-piece micrometer correspond to one division of the stage micrometer. Naturally the way to do this is to observe some number of entire divisions of the stage micrometer that correspond to some number of entire divisions of the eye-piece micrometer, and calculate the value of a single division of the eye-piece micrometer from these data. The figures obtained are the valuation of each division of the eye-piece micrometer scale in terms of the divisions on the stage micrometer. These figures must be determined for each objective used.

These determinations are made by the manufacturers before the microscope leaves the factory, so that practically the user of the microscope, in measuring an object, has simply to observe how many of the divisions of the eye-piece micrometer are covered by the object; divide the result by the number furnished by the manufacturers for the objective used, and multiply the value of one space on the micrometer scale, usually $\frac{1}{10}$ of a millimetre, by the quotient. The draw-tube must be placed at the standard length.

As the eye-piece can be rotated, the micrometer scale may be made to coincide in direction with any dimension of the object, so that the difficulty experienced with the stage micrometer is wholly obviated.

The unit of measurement adopted by microscopists is the micro-millimetre, or $\frac{1}{1000}$ part of a millimetre. It is sometimes represented by mmm; more commonly, and better, by the Greek letter μ. An object which measures .001 of a millimetre in length is said to be 1 μ long; similarly .015 mm. = 15 μ; etc.

To measure the magnification of a camera lucida drawing, place a stage micrometer on the stage with the microscope arranged for drawing. Place two dots on the drawing-paper to mark exactly the limits of the image of one division of the micrometer scale. With an accurate rule measure the distance between the dots. If a micrometer, with an English scale, divided in thousandths of an inch is used, and the image of one division measures one-fourth inch, the magnification is 250; similarly, if a micrometer, with a metric scale, divided in hundredths of a millimetre is used, and the image of one division measures one millimetre, the magnification is 100.

The Dissecting-Microscope. — This accessory is shown in Fig. 12. It is used mainly in the preparation of objects for examination under the compound microscope, or for permanent mounting. As the dissecting-microscope is a simple microscope, the image is not inverted, as is the case with the compound microscope. It is, therefore, easier, especially for a beginner, to work with a specimen under the dissecting-microscope.

Dissecting-needles are a necessary accompaniment of the instrument. They are easily prepared by thrusting ordinary sewing-needles, eye first, into wooden handles about the size and shape of a penholder.

In using the dissecting-microscope, the object to be studied is placed on the stage of the microscope in a watch glass, or other shallow dish with a clear glass bottom, or on a wide slip of glass, either in its natural state, or in a drop of water, or in a drop of some other fluid. It may then be conveniently dissected, treated with staining fluids and reagents, or otherwise specially prepared.

Some workers find the dissecting-microscope a necessity; others accustom themselves to the use of the com-

pound microscope for this work, using a very low power. With practice the difficulties which are at first encountered because of the inversion of the object are overcome, and there is no longer inconvenience.

The Turn-Table. — If one is to mount many objects, and particularly if he has an ambition to do neat work, a turn-table is a necessity. The instrument should be self-centring. It is the most convenient means of centring the object in mounting. The glass slip is placed on the

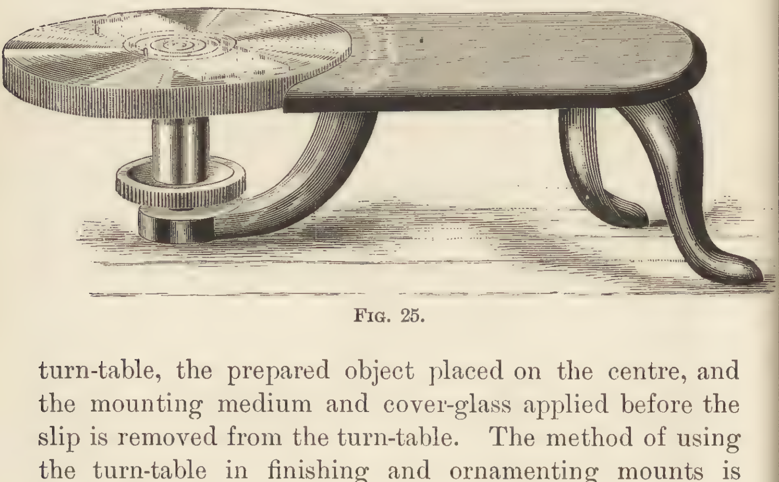


FIG. 25.

turn-table, the prepared object placed on the centre, and the mounting medium and cover-glass applied before the slip is removed from the turn-table. The method of using the turn-table in finishing and ornamenting mounts is described in Chapter VI.

The Section-Cutter. — While, for purposes of studying fresh objects, sufficiently good sections may be made free hand by the method described in Chapter V., if many sections of uniform evenness and thickness are required, a section-cutter, or microtome, must be employed. There are many forms of both. Fig. 26 shows a convenient form of section-cutter to be clamped to the table. If the object is of a suitable degree of firmness to be cut with-

out special preparation, as, for instance, many young plant stems, it is placed in the jaws of the clamp with its upper end even with the upper surface of the section-cutter. By means of the micrometer screw any desired measured thickness of section may be obtained. The sections are cut with a razor, ground flat on one side, held squarely on the glass plates on the top of section-cutter, and drawn across the specimen obliquely. In this instrument, one

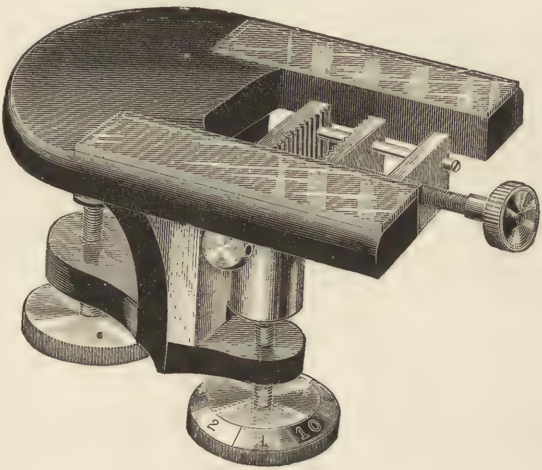


FIG. 26.

complete revolution of the micrometer screw raises the specimen $\frac{1}{50}$ of an inch. The head of the micrometer screw is graduated into ten divisions. If it be turned through one of these divisions, the object is raised $\frac{1}{500}$ of an inch. By turning the micrometer screw through a fraction of one of these divisions, sections of greater fineness may be cut.

The Microtome. — Fig. 27 shows the laboratory microtome of the Bausch and Lomb Optical Company. The prepared specimen is placed in the jaws of the clamp,

which may be adjusted at any height and at any angle by means of the adjustments, *H* and *G*. The lever, *J*, also

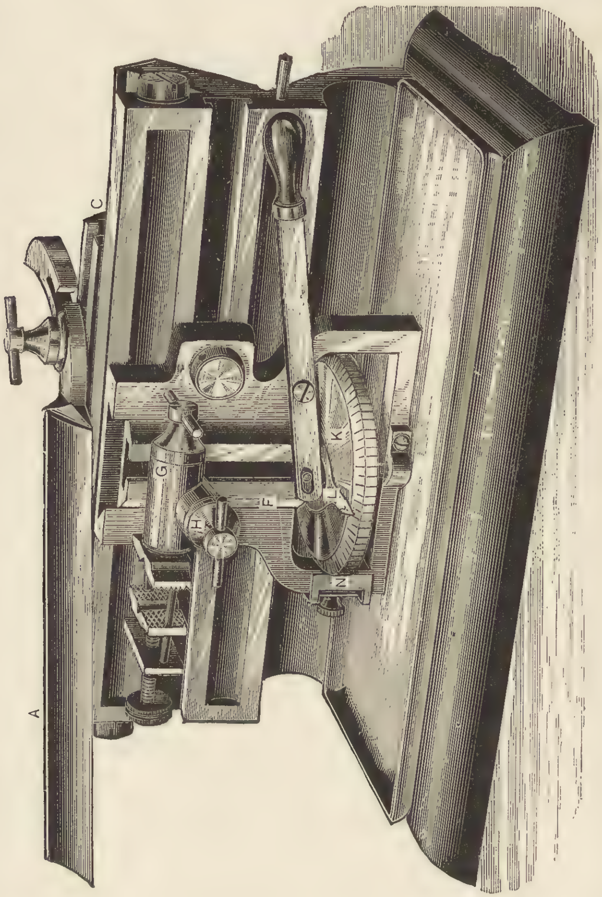


Fig. 27.

furnishes means of raising and lowering the carriage, *F*, which carries with it *H*, *G*, and the clamp. The graduated disk, *K*, is mounted upon a delicately cut screw,

which, by turning *K*, may be brought up to support *F*. The pitch of the screw is $\frac{1}{50}$ of an inch. The disk has eighty divisions in its graduation. By turning the disk through one of these divisions, the clamp carrying the specimen is raised $\frac{1}{4000}$ of an inch. The amount which the disk, *K*, is turned is indicated to the eye by an index, *L*, and to the ear by a piece of spring metal, *N*, which may be adjusted to give a pronounced click as each of the divisions on the rim of the disk passes it.

The knife, *A*, is mounted on a block, *C*, which slides the entire length of the instrument. The knife may be placed so as to strike the specimen at any angle. It is usually adjusted to cut the specimen obliquely.

Methods of preparing hard and soft tissues for cutting with the microtome and the section-cutter are given in subsequent chapters.

The Water-Bath and Oven. — In the processes of preparation of objects for imbedding in paraffin a water-bath is indispensable. A bath may, of course, be improvised. The form represented in Fig. 28 is very convenient. The vessel is capacious, holding a large volume of water. This is a great advantage, as the temperature is under much better control than when a small amount of water is used. The paraffin cup sits in a compartment extend-

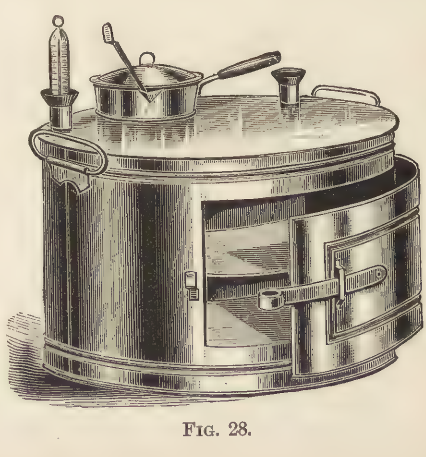


Fig. 28.

ing deeply into the water. A thermometer indicates the temperature. The oven is useful in evaporating volatile reagents from a section, as in removing the clove oil from the collodion fixative used in fixing sections to the glass slip; also in hardening balsam mounts and some kinds of finishing cements. The bath is heated over a laboratory burner or an oil stove. A temperature of between 130° and 140° F. is suitable for most purposes for which the bath is used. After this temperature is reached, the flame is turned low, and with a little experience it is not difficult to adjust it so that the temperature will not vary more than three or four degrees in several hours.

CHAPTER III.

POLARIZED LIGHT AND THE POLARIZER.

The elasticity of the ether in space is believed to be equal in all directions. The same is true of the ether in non-crystalline substances and in crystalline substances of the cubical system. The particles of ether are consequently free to vibrate equally in all directions. In other crystalline substances the elasticity of the ether is modified by the crystalline structure. In some crystals there is one axis or direction about which the molecules are arranged in a uniform manner; such crystals are said to be uniaxial. In other crystals there are two such axes. If a ray of light passes through a crystal along one of the axes, the ether particles are as free to move in one direction as in another; and, except that the light is retarded and to some extent absorbed, which is the case in all dense media, the ray passes through unmodified. If, however, the ray passes through a crystal in any other direction than along an optic axis, the particles of the ether are restricted in their motion in some directions more than in other directions. The result is that the wave movements are mechanically resolved into two sets of vibrations at right angles to each other, and still at right angles to the direction of motion of the ray; but as there is greater freedom of motion in one direction than in the other, the vibrations in this direction are longer than those in the other direction. The effect on the ray is that it is divided into two rays, each caused by vibrations in a single direction.

These rays are both refracted; but as the density of the crystal is different in different directions on account of the difference in structure, the rays are refracted to different degrees, so that they leave the crystal as two separate rays. In some crystals one of these rays is refracted like an ordinary ray of light, and is therefore called the ordinary ray. The other ray is differently refracted in different positions of the crystal, and is called the extraordinary ray. By the unaided eye these rays cannot be distinguished from ordinary light, but when examined by special means they are found to be quite different. Light when thus separated is said to be polarized.

The number of substances that exhibit double refraction is very large; in fact, it is the rule for crystalline substances other than those of the cubical system. Many organic substances also have such differences of structure in different directions that they act in a similar way upon light. The substance that exhibits this property in the highest degree, and the one that is of the most consequence to the microscopist from the fact that the polarizer and analyzer of the microscope are made of it, is Iceland spar, or the crystallized carbonate of lime. This is a uniaxial crystal, the axis joining the blunt angles. If an object is viewed through a crystal of Iceland spar in any other direction than that of the optic axis, it appears double, and if the spar is turned around, one of the images, that formed by

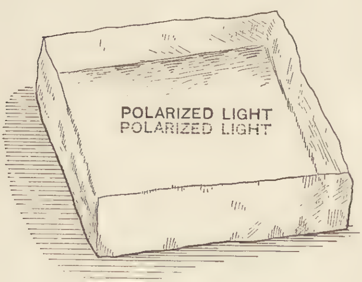


Fig. 29.

the extraordinary rays, revolves about the other. The ray which is most refracted is the ordinary ray. The index of refraction is 1.654. The index of refraction for the extraordinary ray varies from 1.654 to 1.483.

For use in optical instruments, it is desirable to obtain one of these polarized rays entirely apart from the other. This is very ingeniously done in the Nicol prism. In the construction of this prism, advantage is taken of the principle of total reflection, and of the fact that the index of refraction of Canada balsam (1.54) is less than the index of refraction of the ordinary ray, and more than the minimum index of the extraordinary ray. We have already seen in Chapter I. that when a ray of light, in passing from a dense into a rarer medium, strikes the surface at certain angles, it is totally reflected. A rhombohedron of Iceland spar, Fig. 30, three times as long as thick, is cut by a plane *AB*, joining the obtuse angles, the ends being cut off so as to form right angles with the dividing plane. The parts, after being polished, are cemented together with Canada balsam. A ray of light entering the prism from the direction *EF* is doubly refracted at *F*. The ordinary ray, *FK*, meets the surface of the balsam at an angle greater than the critical angle, and is totally reflected along the line *KL*, and thus thrown out of the field of view. The extraordinary ray, *FG*, passes straight through the prism, and after refraction on passing into the air, takes a course parallel to its course *EF* before entering the Iceland spar. By using the Nicol prism, then, we obtain a beam of

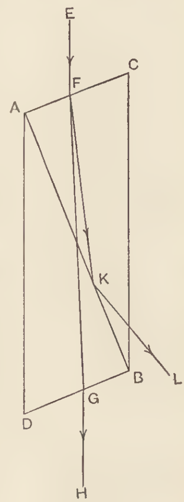


FIG. 30.

polarized light, or light caused by vibrations in a single direction at right angles to the direction in which the ray is travelling.

Two Nicol prisms appropriately mounted constitute the polarizer and analyzer of the microscope. One is mounted to be attached to the sub-stage, and is called the polarizer, Fig. 31. The other, the analyzer, Fig. 32, is screwed into the body of the tube, in the place usually taken by the objective, and the objective is screwed into the lower part of the mounting of the analyzer, so that the analyzer is

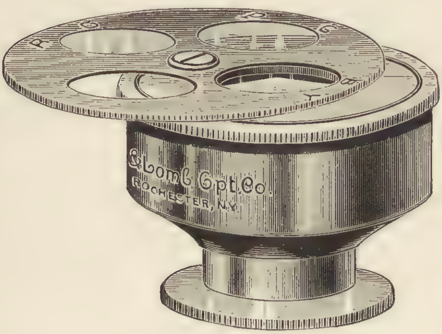


FIG. 31.

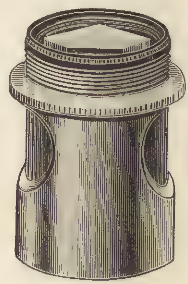


FIG. 32.

between the objective and the body of the microscope. The analyzer is sometimes mounted in a double compartment box arranged to slide in and out in the body over the objective. In one position of the box the light passes through the analyzer. In the other position the light passes through an empty opening. This is a very convenient way of mounting. The analyzer is also mounted to be placed over the eye-piece in some instruments. The only conditions that must be fulfilled are that objects to be examined by polarized light must be

placed between the polarizer and analyzer, and the Nicols must be so placed that the whole of the light can pass through them.

Either the polarizer or analyzer is mounted so as to be rotated about the optical axis of the microscope. In the better grades of microscopes, the mounting of the rotating Nicol has a graduated circumference. When the rotating Nicol is in the same position as the fixed Nicol, the axes of the Nicols are said to be parallel. When the rotating Nicol has been turned through an angle of 90°, the axes are said to be crossed. The effects produced are precisely the same whether it be the polarizer or the analyzer that is rotated. For purposes of explanation, it is less confusing to consider that the analyzer is turned. In the following pages, then, the analyzer will be understood to be the rotating Nicol.

Place the polarizer and analyzer on the microscope, using a low-power objective and eye-piece, and turn the rotating Nicol until the field is well lighted. Observe the reading of the graduated circumference, and turn the Nicol through 90°. The field is now dark. Turn the Nicol through another 90°, and the field is again light. Turn it another 90°, and we have darkness again. Turn it still another 90°, and we have completed the circle and come back to the original position of light.

We will trace a ray of light in its passage from the mirror through the Nicols. As we have seen, the ray is doubly refracted by the Nicol, the ordinary ray being thrown out of the field of view, and the extraordinary ray, which is caused by vibrations in a single direction at right angles to the direction in which the light is travelling, is alone left to pass on. The vibrations which produce this ray are in a definite plane, one which permits them

to pass through the Nicol prism in planes lying in a particular direction. If the analyzer is in exactly the same position as the polarizer, or if it is turned 180° from the position of the polarizer, these particular planes lie in the same direction in both polarizer and analyzer. The vibrations can pass, and the field is light. Turn either Nicol from this position, and the particular planes which allow the vibrations to pass do not correspond in direction in the two Nicols. As the rotating Nicol is turned from the position parallel with the other Nicol, the field gradually grows dark. This is due to the fact that the vibrations strike the second Nicol at an acute angle with its planes of vibration, and are mechanically resolved into two sets of rays at right angles to each other. The rays of one set are in the planes of vibration of the second Nicol, and pass, lighting the field to some extent; the rays of the other set are at right angles to the planes of vibration in the second Nicol, and are suppressed. As the angle at which the rays strike the planes of vibration of the analyzer increases in size, the resolution of the rays becomes less and less, until, when the analyzer has been turned 90°, the rays strike the planes of vibration of the analyzer at right angles. There is now no resolution of the rays, the light cannot pass at all, and the field is dark. Continue to turn the analyzer. The rays now strike its planes of vibration at an acute angle, and are resolved into two sets. At first the amount of the resolution is small, but it increases until the analyzer is turned 90° from the position of darkness. The rays now strike the planes of vibration of the analyzer at an angle of 0°, or parallel to these planes. They are wholly transmitted, and the field is light. Note that this is the position 180° from the first position of light. Again continue to turn the analyzer,

and the field gradually grows dark for the same reasons as in the first case.

Colors in Thin Plates. — Cross the Nicols so as to produce a dark field. Place on the stage of the microscope a thin piece of mica, and slowly revolve it. Four positions of the mica, or two directions at right angles to each other, will be found in which the field remains dark. For all other positions of the mica, the field will be more or less lighted, and if the thickness of the mica plate is right, the light will be colored. The brightest colors are seen when the mica is turned 45° from the position of darkness.

Mica is a doubly refracting substance. When in the positions of darkness, one set of the planes of vibration by which light can pass is parallel with the planes of the polarizer, and the other set with the planes of vibration of the analyzer. It is as though one set of these planes of vibration in the mica were a continuation of the same planes in the polarizer, and the other set a part of the corresponding planes of the analyzer. The light is not further modified, and the field remains dark.

In any other position of the mica plate, the ray coming from the lower Nicol, striking against the mica, is doubly refracted, and passes out of the mica as two rays in directions parallel to each other. As in the case of Iceland spar, one of these rays is more refracted than the other, and must pass through a greater thickness of mica before it emerges. It is consequently more retarded than the other ray during the passage through the dense mica, and comes out a short distance behind the other. If, now, the thickness of the thin plate of mica is right, the retardation may be of such an amount that if the two rays can be brought together so as to interfere, the waves resulting from the interference will be of the right length to produce the sen-

sation of color. But observe that the rays are each caused by vibrations in a single direction, and the directions for the two rays are at right angles to each other. There can be no interference unless the rays can be brought into the same plane. This is what actually happens in the analyzer. Suppose the mica to be in one of the positions which give the brightest color. The two sets of vibration at right angles to each other, coming from the mica plate, strike the analyzer at an angle of 45° with its planes of vibration. Each of the two rays is mechanically resolved into two sets of vibrations at right angles to each other, one set of vibrations from each of the original rays lying in the planes of vibration of the analyzer, the other set from each lying at right angles to the planes of the analyzer. The latter are suppressed; the former pass, and if one is behind the other by half of the wave length of any of the colors composing the white light of which the rays are made up, that color is suppressed through interference, and the color of the transmitted rays is complementary to the suppressed color. The particular color produced depends on the thickness of the mica plate, for, as is well known, the waves that produce the sensation of red light are nearly twice as long as those that produce the sensation of violet light, and the other colors are produced by waves of lengths intermediate between these extremes. Different thicknesses of mica, therefore, cause interference of the waves of different colors, and the transmitted complementary colors are different. If, however, the mica is quite thick, the retardation is such as to suppress by interference portions of the several colors of which the white light is composed, so that the effect is simply to reduce the intensity of the white light that is transmitted.

Without changing the positions of the polarizer and analyzer, place pieces of mica of different thicknesses upon the stage, and in each case turn the piece until the brightest color is produced. It is at once seen that, within certain limits of thickness, each different piece produces a different color, but that pieces beyond a certain thickness do not cause color.

Complementary Colors. — Cross the Nicols, place a plate of mica on the stage, and turn it until the field is dark. If the microscope has a graduated revolving stage, the next step is easy; if not, the measurements may be taken with sufficient accuracy with a protractor. Revolve the mica until the field is brightest. It will be found, by accurate observation and measurement, that the mica has been turned through 45°. The two rays coming from the mica now strike the analyzer so that the vibrations of each form an angle of 45° with the planes of the analyzer. As we have before seen, each vibration is mechanically resolved into two at right angles with each other, one set of vibrations from each of the original rays lying at right angles with the planes of vibration of the analyzer, and so not able to pass, the other set of vibrations from each of the original rays lying in the same planes with the planes of vibration of the analyzer. The latter pairs of vibrations pass, interfere, and produce waves of the right length to cause the sensation of color. Observe what happens when the analyzer is turned through 90° from this position. The pairs of vibrations, which before could pass, now strike the analyzer at right angles to its planes of vibration, while the pairs, which before were suppressed, now lie in the same planes with the planes of vibration of the analyzer, pass, interfere, and cause a color.

Observe that the sum-total of the vibrations which

caused the last pairs of rays, plus the total vibrations which caused the former pairs, originally produced white light. We should, therefore, expect that the color produced in the last instance, added to the color produced in the first, would produce white light; in other words, that the colors would be complementary. We find that this is the case.

If we turn the analyzer through another 90°, its planes of vibration lie in the same directions as in the first instance, and the same color is seen. Turn the analyzer through another 90°, and the complementary color must again result.

The Dark Cross. — Cross the Nicols, and place upon the stage a slide of sulphonal crystals prepared by the method described in Chapter X. The light is more or less restored, and black crosses, the arms at right angles to each other, are seen. Bring the point at which the arms of one of these crosses intersect to the centre of the field, and revolve the specimen on the stage. The cross will be seen to be stationary. It shows the position of the planes of vibration in the Nicols, one arm of the cross lying in the planes of the polarizer, the other in the planes of the analyzer. It appears because there is no polarization in these two directions, the light being simply absorbed. Other portions of the crystal are colored because there is polarization in all other directions.

Turn the analyzer 90°, and a white cross takes the place of the black one, the colors of the intervening spaces at the same time changing to their complementaries, so that there is an appearance of a revolving cross changing color as it revolves. There is still no polarization in these two directions, but the light is now transmitted.

A full explanation of these phenomena would involve a

discussion of elliptical polarization, which would require too much space for a proper presentation here. Suffice it to say that these appearances are similar to the phenomena produced by uniaxial crystals cut perpendicular to their optic axes, and viewed in convergent polarized light. In the crystals of sulphonal the point of intersection of the arms of the cross is a point on the axis of the crystal. From this point the crystallization proceeded laterally in all directions. This may easily be verified by watching the formation of the crystals when the slide is prepared. These crystals are, then, similar in structure to sections of uniaxial crystals cut perpendicular to the principal axis. Such sections of quartz, emerald, mica, and other uniaxial crystals show the cross when viewed in convergent polarized light. Crystals of salicine and other chemical salts which form from centres, as do the sulphonal crystals, on account of the peculiar arrangement of their molecules around the axis, show the cross in plane polarized light. Starches, for a similar reason, show a more or less distorted cross, very characteristic and interesting, radiating from the hilum.

Uses of Polarized Light. — As has already been seen, the particular color produced by the interposition of a thin film depends upon the thickness of the film. If the film is of varying thickness, or of varying composition, different colors are produced simultaneously. In the case of many chemical crystals the slight differences in thickness give rise to most beautiful combinations of colors, rendered still more striking by the changes to complementary colors as the analyzer is turned. Many crystals that are almost, if not quite, invisible in ordinary light are made plainly visible by using polarized light; in some cases revealing most delicate and beautiful forms. It is hardly

possible to use too strong language in describing the fascination felt in watching the process of crystallization under polarized light, or even in examining crystals already formed and permanently mounted.

In the case of rock sections the different minerals of which the rock is composed affect the light differently, and the different effects produced are important aids in distinguishing and determining the composition of the rock.

So, too, in the case of many common objects, there are sufficient differences in structure to polarize the light in greater or less degree. The structure of the object is usually made much plainer in some positions of the analyzer. Polarized light is, therefore, a valuable means of effecting special illumination of some classes of objects. It is always well to try polarized light on any very transparent object. The results thus obtained are in many cases equally surprising and interesting.

A few of the large number of polarizing substances are the following: thin and transparent cellular tissue of plants; plant hairs, which often yield most striking effects; starches; the silicious cuticle of plants; fibres of cotton, wool, and hemp; crystalline deposits in plant tissues and sections; muscular tissues of animals; animal hairs; sections of bone, horn, and similar substances; all crystalline substances except those of the cubical system. It would not be possible, nor is it desirable, to give a complete list. Even when an object does not distinctly polarize, the modification of the light effected by turning the analyzer aids materially in bringing out the less distinct portions of the structure.

Substances which are not of the right thickness of themselves to polarize often give good effects when a film of selenite or mica is used with them to increase

the thickness. Such a film should form a part of the polarizing outfit. It may be introduced anywhere between the polarizer and analyzer. A single film is sometimes mounted in the fitting of the polarizer, or several films of different thicknesses are so mounted on the polarizer fitting that any one may be interposed at will, as is shown in Fig. 31. The film is sometimes mounted in a brass plate to be laid upon the stage. It is then called a selenite stage. Sometimes it is mounted in a small metal plate to be inserted in an opening made for it in the body of the microscope just above the objective, but, of course, below the analyzer.

A well-selected mica film, mounted in Canada balsam on an ordinary glass slip, and covered with a thin cover-glass to prevent its becoming scratched, serves the purpose well.

Single-Refracting and Double-Refracting Objects. — It is evident from what has already been said that polarized light affords means of determining whether a substance is singly refractive or doubly refractive. If a singly refractive substance, as, for instance, a piece of ordinary glass, or a crystal belonging to the cubical system, as crystals of potassium iodide, potassium bromide, or common salt, is placed upon the stage with the Nicols crossed, the field remains dark, nor are color effects produced when the analyzer is turned. Such substances are said to be optically isotropic or monorefringent.

If a doubly refractive substance, as, for instance, any chemical crystal not belonging to the cubical system, or any other of the substances already mentioned as polarizing substances, is placed upon the stage, the Nicols being crossed, the light is restored and color effects are produced, when the analyzer is turned. Such substances are said to be birefringent or anisotropic.

CHAPTER IV.

INSTRUCTIVE PRACTICE IN MANIPULATION.

THE beginner will do well to make a careful study of the microscope before attempting practical work. With this end in view, he may put himself through a course of training in microscopical gymnastics. The knowledge gained will be ample reward for the trouble. One or two slides of diatoms, to be purchased at twenty-five cents each of various dealers, will afford means for very instructive study of the instrument. A slide of the diatoms called *Arachnoidiscus Ehrenbergii* and one of *Pleurosigma angulatum* will serve the purpose. In the lack of these slides the scales of a moth, obtained by pressing the wing of a moth to a cover-glass, or a little potato starch dusted on a cover, may be used. Invert the cover on a glass slip.

Place the slide of *Arachnoidiscus Ehrenbergii* on the stage of the microscope and focus sharply upon it, using the *A* eye-piece and the $\frac{3}{4}$-in. objective. In focussing, do not at first look through the instrument, but place the eye in position to watch the approach of the objective to the glass slip on the stage, and slowly move the body of the microscope down by means of the coarse adjustment until the front lens of the objective is as near its working distance from the glass slip as can be estimated; in this case about three-fourths of an inch. Now look through the instrument, and the outlines of the object should be more or less visible if the object is properly centred on the stage. If nothing is seen, move the glass slip about to

centre the object more perfectly. If it is now necessary to use the coarse adjustment to change the focus, focus up only. If the object is not thus brought into view, turn the body down again with the coarse adjustment while watching the approach of the objective to the object as before. When the object is brought into view, focus up and down with the fine adjustment to obtain a more perfect image. Recall what was stated in Chapter I. about the influence of the thickness of the cover-glass on the clearness of the image. If the thickness of the cover-glass is known, adjust the draw-tube to the required length. If the thickness of the cover-glass is not known, after getting the sharpest image possible with the draw-tube at the standard length, try the effect of longer and shorter lengths until the image is clearest.

See that the field is properly lighted. Direct sunlight must not be used. The diffused sunlight from a north window, or the light reflected from a cloud or white wall, is regarded as the best light. The light from an ordinary kerosene lamp is a very suitable artificial light.

Place the mirror in central position, and use first the plane and then the concave side. Use each of the openings in the diaphragm in succession with each mirror. Focus up and down occasionally with the fine adjustment. Practise these manipulations until the arrangements are found which show the markings on the diatom most perfectly. It will be found that the best effects are obtained when the field is lighted with a subdued light that is very pleasant and comfortable for the eye. A glare of light should always be avoided. The light should be sufficient, but not too intense. If the light is thus properly regulated, and suitable precautions are exercised not to use the eyes to the point of fatigue, the

eyes will be strengthened rather than injured by microscopical work. Just as judicious exercise strengthens the body, so judicious use of the eyes improves them. If one makes a practice of reading in the glare of the sun or other light, his eyes will soon be injured. So too, if he uses the microscope with glaring light, his eyes will be injured. The correct modulation of the light is a matter of very great importance, and one that is too often overlooked even by those who have had considerable experience with the microscope. The light from the concave mirror is more intense than that from the plane mirror, and is better adapted to the higher power objectives and eyepieces, which absorb more light than do the lower powers. In using the concave mirror, slide it up and down on the mirror bar until a bright circle of light from half an inch to an inch in diameter is projected upon the slide. This circle of light is best seen when the slide is shaded from other light.

After noting the effects obtained from each of the above arrangements, move the mirror about 10° to the side away from the light, adjust it to the light, and again go through the same practice manipulations, carefully observing differences of effects. Then move the mirror another 10° to the side, and again repeat the manipulations. Continue to move the mirror to one side 10° at a time, and to repeat the above manipulations as long as good results can be obtained. Having observed the effects for each position of the mirror with the draw-tube closed, place the draw-tube at different lengths. Notice the slight change of focus necessary and the effect on the image. Determine what length of draw-tube gives the best results.

Remove the *A* eye-piece and put in its place the *C* eye-piece, and practise the manipulations again. When

some degree of skill has been acquired in manipulation, the $\frac{1}{5}$-in. objective may be substituted for the $\frac{3}{4}$-in., and the slide of *Pleurosigma angulatum* for the *Arachnoidiscus Ehrenbergii* as a more suitable object for this objective. In focussing the $\frac{1}{5}$-in. objective the precaution to watch it as it is brought to its proper working distance, about $\frac{1}{5}$ of an inch from the slide, must be carefully observed. On no account allow the front lens to come in contact with the cover-glass, or both objective and cover-glass may be injured. With both the *A* and the *C* eye-pieces repeat the manipulations described above until the best results possible are obtained.

Patient practice of these manipulations at intervals for a few weeks will give one a knowledge of the instrument, and an ability to handle it in practical work that cannot be gained by years of heedless use.

Examination of Opaque Objects. — Place a glass slip upon the stage, and on it any opaque object, as, for instance, seeds of grasses or flowers, a plant leaf, grains of sand, or an insect. If the mirror can be swung above the stage, arrange the concave mirror so as to focus the light upon the object. This should be done roughly before looking into the microscope. With the coarse adjustment move the objective to about its working distance. Then looking at the object through the microscope, focus up and down with the coarse or fine adjustment, as occasion may require, always remembering to guard against bringing the objective too near the slide or specimen. Change the position of the mirror to illuminate the object better. If the mirror cannot be used thus, a bull's-eye condenser may be used to focus the light upon the object, the mirror being put in such position that light reflected from it will not interfere with the illumination.

Air Bubbles. — Many curious and ridiculous blunders have been made from misinterpretations of the appearances of air bubbles. To guard against such errors, air bubbles should be made the object of special study. A drop of saliva, or soapsuds, or of mucilage well beaten up on a slide with the point of a knife blade, have been suggested as suitable means of easily obtaining the bubbles for study. Study the bubbles both without and with a cover-glass, which should be placed lightly over the mass. Focus up and down, using both central and oblique light. Once studied in this way, the air bubble will ever after easily be recognized. Observe that air bubbles afford a most excellent means of determining when the light is central.

Oil Globules. — Oil globules have also led to errors of interpretation. They are liable to occur in preparations of animal or vegetable tissues. To form them for study, beat up a small drop of any oil with a drop of water on a slide, cover lightly, and study them in the same manner as the air bubbles.

Fibres and Hairs. — Fibres of cotton, wool, silk, and hemp, and hairs of different kinds, are liable to find their way into preparations from the air of the room, or from lack of care during the processes of preparation. It is well to make a special study of these and similar objects. Examine them dry, also in water or glycerine, or in some oily medium, as oil of turpentine, or oil of cloves, covering with a cover-glass. Examine in a similar manner any other objects which may suggest themselves as likely to find their way adventitiously into a microscopical preparation.

Currents and the Brownian Movement. — Grind up a little solid carmine, or any very finely powdered mineral sub-

stance, in a drop of water on a glass slip, cover with a cover-glass, and place the slip on the stage of the microscope. Incline the stage, and watch the current movement of the particles. Then place the stage in the horizontal position, and wait a few minutes for the currents to cease. A peculiar, jerky motion of the finer particles may now be observed as they dart about among the larger masses. This is the so-called Brownian movement, or Pedesis. The study of these phenomena may prevent serious errors of interpretation.

Practice with Immersion Objectives. — On beginning to use an immersion objective, practice work is of especial importance. The slide of *Pleurosigma angulatum* is still a good object to use, as the $\frac{1}{5}$-inch objective has left much unseen. With this slide on the stage, place a small drop of water, if the objective is a water-immersion objective, or of homogeneous immersion fluid, if the objective is a homogeneous immersion objective, on the front lens, or on the centre of the slide, the microscope being placed in the upright position. Watching the objective, run it down by means of the coarse adjustment in the manner previously described, until the drop of fluid forms a connection between the front lens and the cover-glass. The drop should be just large enough to fill the space between the lens and the cover-glass. After the connection is made, the microscope may be inclined, if desired. If a non-adjustable objective is used, and the thickness of the cover-glass is known, the draw-tube should be adjusted to give the correct tube length according to the cover-glass gauge. When the tube length can thus easily be obtained, much has been gained. We have now simply to light the field well by means of the sub-stage condenser, remembering that as much depends upon correctly focussing the con-

denser as on focussing the objective, and obtain the correct focus of the objective by means of the fine adjustment. One should remember always to focus up and not down, or both the cover-glass and objective will be endangered.

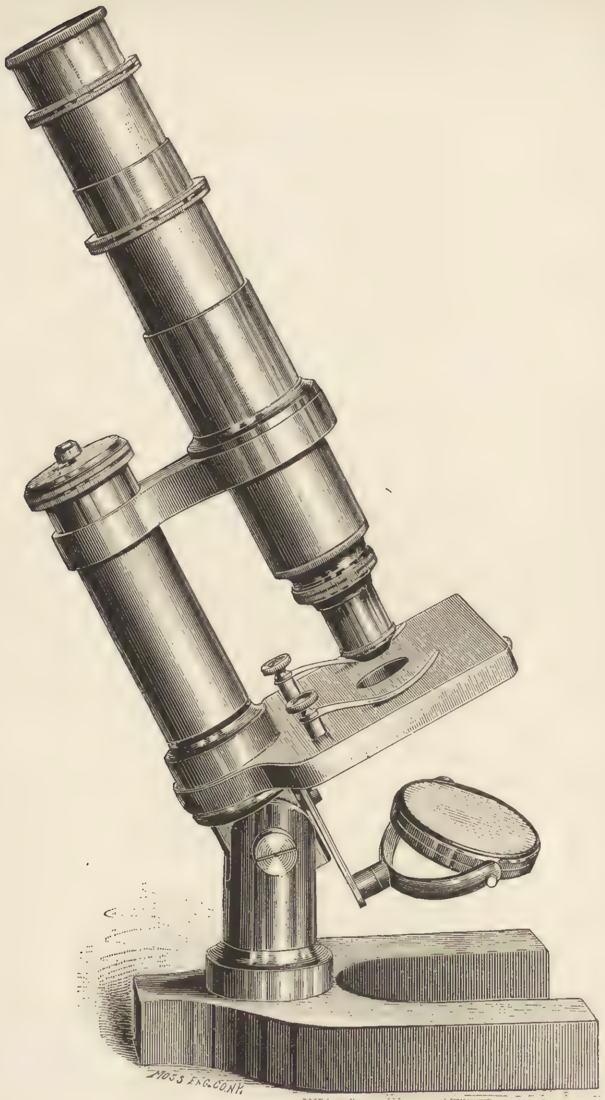
If the objective has collar adjustment, and the thickness of the cover-glass is known, the collar may be set at once at the proper mark, remembering that the objective should be set at the medium point for cover-glasses of the standard thickness adopted by the makers of the objective. If the cover-glass is thinner than the standard thickness, the milled collar should be turned towards the lowest point. If the cover-glass is thicker than standard thickness, the milled collar should be turned towards the highest point, the amount of the turning being estimated by the amount of deviation from the standard thickness of cover-glass.

If the thickness of the cover-glass is not known, the collar should be set at the highest point, that is, for thick covers, when the connection is first made with the cover-glass. Now with one hand on the milled ring of the collar adjustment, and the other on the fine adjustment, and while looking into the microscope, turn the collar adjustment through part of one of its divisions, and gently focus up and down with the fine adjustment. Repeat this manipulation until the most perfect image attainable has been obtained, remembering to test the focussing of the sub-stage condenser from time to time by moving it up and down.

After obtaining the best effects possible with central light, oblique light should be used. If the sub-stage condenser is provided with a diaphragm, make the diaphragm eccentric; that is, move it a little to one side. After noting the effects, by successive steps make the diaphragm more and more eccentric as long as good results

can be obtained. Then, in like manner, move the diaphragm in the opposite direction. If there is no diaphragm, obtain oblique light by means of the mirror by moving it away from the light a few degrees at a time in the same manner as when using the low power objectives.

It cannot be stated too emphatically that a large amount of this practice manipulation should precede any attempts to make practical use of immersion objectives. The ability to bring out the full power of these objectives can be attained only by patient perseverance and the intelligent study of the effects produced by manipulation. It hardly seems necessary to waste time in describing the results which should be obtained. The observer will have very little doubt about his success when he obtains clear-cut images, and he should not be satisfied until he does this.



CONTINENTAL MICROSCOPE.
Made by Bausch and Lomb Optical Co., Rochester, N.Y.

FIG. 33.

CHAPTER V.

METHODS OF STUDYING FRESH OBJECTS.

THE microscope is but an aid to the eye, and the eye an aid to the brain behind it. Something more than the mere collection of objects and multiplying the number of slides in a cabinet should be the aim of every owner of a microscope. The highest use of the instrument is in following out definite courses of investigation. In the pursuit of such investigations the examination of a very large number of different specimens is necessary to obtain clear ideas of structure, and give the brain material with which to work. It would not be possible, nor would it be desirable, permanently to preserve all the specimens examined. The methods of working will of course differ with different lines of investigation.

It is not the purpose here to furnish directions and descriptions for detailed and comprehensive work in any department. Volumes would be required for this, and excellent manuals already exist for the different departments of investigation. It will be convenient for the purposes of this chapter to consider some methods of working with vegetable and animal specimens, and to illustrate the methods by particular descriptions in a few cases. For methods of preparing vegetable and animal specimens for permanent mounting the reader is referred to Chapters VIII. and IX.

In the investigation of these specimens, a razor ground flat on one side, a supply of glass slips and cover-glasses,

some dissecting-needles, and various inexpensive chemicals and stains, formulas for which will be found in the Appendix, are necessary. Glass slips three inches by one and a half are to be preferred for this purpose to the ordinary slips, as they present a broader surface to hold the reagents used. For many of the processes a dissecting-microscope is a great convenience. This is most needed when many dissections are to be made. If this instrument is not at command, use the lowest power of the compound microscope.

The microscope is best kept in the upright position to prevent the specimen from slipping out of the field of view, to prevent currents due to gravity in the fluid media employed, and to prevent the reagents from injuring the microscope. Neatness in all operations is the mark of a skilful workman. In case any of the reagents used accidentally come in contact with the microscope, they should immediately be removed. The reagents should not be allowed to flow over the upper surface of the cover-glass, as they are liable to come in contact with the front lens of the objective. Especial pains should be taken to prevent this, and in case they do touch the objective, it should immediately be cleaned with great care.

In these practical studies air bubbles and oil globules are very sure to be met with, and it may be well to recall or repeat the studies already made of these objects.

Vegetable Specimens.

For purposes of study vegetable substances are usually examined in water. Some substances require no special preparation. Any of the minute and transparent plants, either those growing on land or those growing in the

water, may be placed on the centre of a glass slip in a small drop of water, a cover-glass laid over the specimen, and the examination made. Care should be exercised not to use so large a drop of water that it will run out from under the cover-glass. Enough only should be used to fill the space between the cover-glass and the slip. If more is added, it is liable to run on to the microscope. In any case the work is slovenly. An excess may be removed by touching it with a piece of blotting paper or of filter paper.

Microscopic Water Plants. — The light green stringy masses found floating in the water or attached to submerged objects in ponds and ditches in summer afford good material for examination. These plants belong to the family of Zygnemaceæ. They are unicellular, but the cells are joined in large numbers so that the plant appears to be multicellular. It is easily recognized by its slippery feeling when a mass of it is taken in the hand, and from the fact that each filament is without branches. In young cells the greenish yellow chlorophyll granules are quite evenly distributed throughout the cell. At more advanced stages the chlorophyll collects in some species in a line running lengthwise through the centre of the cell, often terminating in star-shaped or globular knobs, so that each cell contains a dumb-bell-like figure. These knobs are sometimes left isolated by the breaking down of the connecting line of chlorophyll granules.

In other cases the chlorophyll collects into spiral bands, differing in number in different species, the bands giving these species their name, *Spirogyra*. In favorable cases conjugation between the cells of two adjacent-lying filaments affords opportunity for much interesting study. June or July is the most favorable time to observe the

conjugation. From this yoking together of the cells in conjugation the whole family takes its name, Zygnemaceæ. There are many varieties in the family, any one being an interesting object for study.

In examining this plant, observe that the cells are all alike, and, being joined end to end, form filaments of great length, but of uniform diameter throughout. As the plant approaches maturity, the bright yellow-green changes to a dirty brown. When at this stage, it is in a particularly favorable condition to exhibit some of its distinguishing features.

Attached to the cells of the *Spirogyra* are frequently found numerous spherical, or nearly spherical, bodies of a reddish color. They are the *Vampyrella spirogyræ*, belonging to the Monerozoa, one of the lowest types of animal life. They have attacked the *Spirogyra* to feed on its chlorophyll.

Yeast. — Yeast, the *Torula cerevisiæ*, is another plant which furnishes interesting material for examination. Dissolve some sugar in water, and add a little yeast. When it is seen that the yeast is working, place a drop on a glass slip without a cover-glass, and examine with a low power. Then cover, and examine with a power of four or five hundred diameters. The yeast is seen to be made up of very transparent spherical and oblong cells of the same kind, but of varying sizes. Some will no doubt be seen with minute cells attached, showing the characteristic mode of propagation by budding.

Other Studies. — In a similar manner, any other substances which are sufficiently thin to allow light to pass through them may be examined. Pollen grains are thus easily examined, and are very attractive objects. Their forms are almost infinite in number and variety. The

spores of ferns and mosses, and the numerous varieties of fungi, afford abundant material for examination. Of the fungi, mention may be made of the green mould which forms on bread, old leather, and numerous other substances. It is the *Penicillium glaucum*. Another mould, the *Mucor stolonifer*, is easily grown on a piece of bread kept moist under a bell-jar or tumbler for two or three days. It will be recognized by its fine, thread-like stalks, each having a round head, and by the stolons connecting the stalks. In examining either of these fungi, treat different specimens with hæmatoxylin and carmine stains, and with iodine.

The epidermis of leaves and stems may in many cases be scraped off with a knife, or torn off with forceps, and without further preparation be placed under the microscope in a drop of water. The epidermal cells, stomata, and hairs, are thus readily obtained for examination from many plants.

Maceration and subsequent teasing or tearing the specimen to pieces by means of dissecting-needles is a very important means of reaching the minute internal structure of plants. The maceration may be effected by soaking the specimen for some time, or by boiling it a few minutes, in water. It may be effected more quickly and thoroughly by treating with a warm, strong solution of caustic potash, or by placing the specimen in a test-tube with a few crystals of potassium chlorate and enough strong nitric acid to cover them (Schultze's Macerating Mixture). Strong hydrochloric acid is also a good macerating fluid. After the action has continued for a short time, the specimen should be washed thoroughly in water, transferred to a glass slip, and torn to pieces with the dissecting-needles under the dissecting-microscope or a low power of the compound microscope. Spiral and scalariform vessels are

easily obtained by this process from the leaf stalks of rhubarb and of ferns, the stem of banana, and other sources. The cell structure of some tissues is also revealed by this treatment much better in some respects than can be done by sections.

Vegetable Sections. — To obtain sections of vegetable specimens, a piece is held firmly in the left hand between the thumb and second joint of the forefinger, the blade of the razor is rested on the second joint of the forefinger as a guide, and drawn towards the person, resting both hands against the breast to steady them. The thumb of the left hand should be kept depressed to guard against injury. Keep the specimen and the blade of the razor wet with water, and when the section is cut, dip the razor in a glass of water to float the section off. Small or soft objects may be held between pieces of pith, carrot, or potato. Hard specimens may be made soft enough to cut by boiling for a few minutes in water. The hard woods yield to this treatment. For purposes of study it is not always necessary that large, even sections be cut. Indeed, an uneven section is sometimes more valuable than an even one for this use. Some parts near the edges will show extreme thinness, while other parts often give a better idea of the relations of the cells to one another. Ordinarily the rule for thinness is to cut to the thickness of one row of cells. It is best to cut quite a number of sections of each specimen.

For study, a section should be transferred from the glass of water to a glass slip with a small drop of water. A camel's-hair brush is the best section-lifter. The section is covered with a cover-glass and is ready for examination. While water is ordinarily the best medium for the examination of vegetable substances, there are some cases where

it produces an undesirable change in the tissues of the specimen, causing the cell contents or the cell walls to swell. Other reagents then become desirable. Strong alcohol, by extracting water, causes a contraction of the cell wall and contents. More dilute alcohol is, in many cases, a suitable medium to maintain the tissue in its normal condition.

It is often desirable to watch the changes which the specimen undergoes under the action of a reagent. It may be desired, for instance, to remove water from the specimen and introduce alcohol. This is best done without removing the specimen from the slide, or even removing the cover-glass from the specimen. The slide still remaining under the microscope, by means of a pipette (a glass tube drawn out to a fine point) hold a little alcohol near one side of the cover-glass, and at the opposite side hold a piece of blotting paper, or better, good filter paper. The water is drawn out from under the cover-glass by capillary attraction, and the alcohol takes its place. By repeating the operation several times the water is entirely removed and alcohol substituted. In the same way any reagent or stain may be introduced, or the specimen may be washed free of a reagent by means of water.

It often happens that a specimen is too opaque to be well studied, even when the section is very thin. In such cases glycerine is a valuable medium for the examination. If the specimen is already mounted in water, the water should be removed and the glycerine introduced in the manner just described. The changes which the specimen undergoes during the process afford excellent opportunities for studying the object. Opaqueness may also be remedied by bleaching the specimen before it is mounted. Sodium or calcium hypochlorite is a good bleaching agent.

A considerable volume of the fluid should be used, and the specimen should remain in it for some hours. If it is desired to render the specimen more transparent during the progress of the examination, a moderately strong solution of caustic potash may be run under the cover-glass. The first effect of the caustic potash in some cases is to turn the section black. This may be remedied by washing out the caustic potash with water, and running under a very weak solution of hydrochloric acid. The specimen soon becomes transparent. The acid should now be washed out with water. In the more obstinate cases it may be necessary to repeat the treatment with caustic potash several times before the desired effect is produced.

In similar ways the changes which the specimen undergoes when treated with different chemical solutions may be studied. It is usually best to add the reagent in small amounts at first, and to watch the changes as the strength is increased. Any of the staining solutions for which formulas are given in the Appendix may be thus employed. The use of different stains on the same section, or on different sections of the same specimen, tends to reveal varied points of structure. The study of fresh sections in this way is a valuable preparation for the correct use of stains for permanent mounts.

Starch. — Under the microscope starch granules show a distinct point, usually situated near one end, called the hilum, and an appearance of being made up of lamellæ, or plates. The test for starch is a solution of iodine. Iodine dissolved in absolute alcohol gives a brown color to dry starch. If any moisture is present, the color is blue. As an ordinary test for starch, iodine dissolved in a watery solution of potassium iodide may be used. If a little

of this is run under the cover, the characteristic blue color of the iodide of starch appears.

The changes caused in the starch granule by the gradual addition of a strong solution of caustic potash are instructive. As the caustic potash is run under the cover-glass, the starch granule is seen to swell, and the hilum and laminations become more distinct. Soon the lamination becomes less distinct as the granule becomes more glassy and transparent. Dilute chromic acid is another reagent which renders the stratification more distinct.

Under polarized light the starch granules are brought distinctly into view, each with its characteristic black cross. A good specimen for the study of starch granules *in situ* is a section of a potato taken from just below the skin. A little of the juice of a potato, or the flour obtained by grinding to a powder a pea, bean, kernel of corn, wheat, or oats, also affords good material for study.

Cellulose. — Iodine imparts a yellowish brown color to cellulose, the fundamental material of the cell wall. If a little strong sulphuric acid is run into the specimen, the color is changed to blue. The best reagent for detecting cellulose, however, is Schultze's reagent or chlorzinc iodine. This is made by dissolving metallic zinc in hydrochloric acid, evaporating to the consistency of syrup with a piece of undissolved zinc remaining, and adding as much potassium iodide as will dissolve. Finally iodine is added until a deep reddish brown color is produced. This reagent colors cellulose violet.

Another useful reagent for the study of cellulose is ammoniacal cupric oxide. It is prepared by adding caustic soda or caustic potash to a strong solution of copper sulphate as long as a precipitate is formed. Filter, and wash the precipitate in water that has been boiled. Place the

greenish mass in a bottle, and add just enough strong ammonia to dissolve it. When properly prepared, fibres of cellulose swell up and dissolve in it. To test the reagent, see if fibres of cotton are so acted on by it. It must be kept in the dark.

Lignified Cellular Tissue. — Aniline chloride dissolved in ten parts of alcohol and one part of hydrochloric acid imparts a yellow tinge to lignified cellular tissue. Aniline sulphate dissolved in ten parts of alcohol, or of water, and one part of sulphuric acid also gives a yellow color to lignified cellular tissue. Lignified cellular tissue turns yellowish green if treated with a solution of carbolic acid crystals in warm hydrochloric acid and subsequently exposed to the direct sunlight. A strong solution of chromic acid readily dissolves cell walls. In weaker solutions it causes the cell wall to swell. It is therefore a valuable reagent in studying cellular tissues. Schultze's reagent imparts a clear yellow color to lignified cellular tissue, and brings it into prominent view. If this reagent is allowed to act on the specimen for some hours, the result is more decided. Objects will keep in good condition in this solution for a long time.

Protoplasm. — Protoplasm is a viscid, transparent fluid often containing finely divided dark particles, occurring in the younger cells of plants. It is the living part of the plant. It is best studied in the hairs of nettles, the hairs of very young shoots of the pumpkin, squash, or cucumber, and the hairs upon the stamens of the cultivated spiderwort, *Tradescantia Virginica.* If some of these hairs are examined in a drop of water under *high* power, the moving currents of the protoplasm are visible. It is often necessary to warm the slide slightly to cause the movement to start. In studying the protoplasmic movements, remove

the water, and run a little glycerine under the cover-glass in the usual manner, and observe the effects on the protoplasm. The action of alcohol may, in like manner, be observed. Strong alcohol kills the protoplasm, which is then much more readily acted upon by staining fluids. A nucleus, containing a nucleolus, may be seen with a high power, in the cells of successful preparations, suspended by threads, and carried about in the cell as the protoplasm is acted upon by the reagents. If tissues containing protoplasm are treated with a thick syrup of cane sugar, and afterwards with strong sulphuric acid, the protoplasm is slightly colored red. Protoplasm also absorbs iodine from solution, and becomes reddish brown.

After some experience has been acquired in the study of protoplasm in plant hairs, the various reactions for it may be applied to the young cellular tissue found at the growing points of stems, roots, and leaves. It is to be remembered that protoplasm can be studied best in young cells. As the cells increase in age, cavities, or vacuoles, are formed, and the protoplasm is pushed close up to the wall. If such a tissue be treated with a somewhat strong aqueous solution of common salt, the protoplasm is caused to contract and becomes more easily visible. In still older cells the protoplasm wholly disappears. In very young tissues the application of a stain — hæmatoxylin is especially good — often brings out a network of delicate fibres in the protoplasm.

Chromatophores. — The green color of plants is due to minute spherical or polygonal shaped particles imbedded between layers of protoplasm. When viewed under the microscope, they appear to be suspended between the cell wall and the vacuole, or sap cavity, in the central part of the cell. When treated with alcohol for some time,

the color disappears, but the forms of the granules are still faintly visible as colorless bodies. The green pigment is called chlorophyll; the granules that bear the pigment are known as chlorophyll bodies. Other pigments give the characteristic colors to colored plants. Chromatophore is a general name for the bodies that bear the various colors. It is characteristic of chromatophores that starch granules are developed in them. It is not always easy to see these without special treatment. Cut a green leaf into several small pieces, and place the pieces in a drop of water under the microscope. The chlorophyll bodies are liberated, and from some of them the enclosed starch granules are set free. Exposure to direct sunlight for several hours previous to the examination will render success more certain. Treat with iodine solution, and the starch shows the characteristic blue color, while the uninjured chlorophyll bodies take on a brown color. The enclosed starch granules may also be demonstrated by treating material which has lain in alcohol for some time with caustic potash to cause the starch granules to swell, and afterwards treating with iodine solution to produce the blue color. It is to be noted that the origin of starch granules is in the chlorophyll bodies. As the chlorophyll bodies increase in age, they gradually become more and more transparent until they disappear, and the matured starch granules are left uncovered in the cells of the plant.

The prothallia of ferns, which are always found growing on the plant-pots in hot-houses where ferns are cultivated, are particularly favorable specimens in which to study chlorophyll. The Zygnemaceæ are also good material for study. Keep the specimens in the sunlight several hours, treat with iodine, and the masses of chlorophyll will be seen to be surrounded with starch granules.

Plant Crystals. — Deposits of crystalline earthy matters are of very common occurrence in the cells of plants. The crystals most commonly take the shape of a prism, an octahedron, or of needles. Some can be found in almost any plant. The cuticle of the onion shows fine examples of the prismatic and octahedral crystals of the oxalate of lime. Similar crystals may also be found in the bark of the rose around the bases of the prickles. Raphides, or needle-shaped crystals, of oxalate of lime are abundant in rhubarb, especially in the root, and in the epidermis of the bulbs of tulips and hyacinths. The petioles of begonia contain octahedral crystals of the same salt. Tubers of the dahlia which have been in alcohol for some days show somewhat spherical-shaped crystals of inulin. During the examination for inulin allow nitric acid slowly to replace the water.

Owing to the difficulty of isolating the crystals from the cellular tissues the chemical composition is not known in many cases. It is known, however, that crystals of the oxalate of lime are most common. The carbonate of lime is the second most common crystal found. These are easily distinguished by means of acetic and hydrochloric acid. If acetic acid is applied to the section, crystals of calcium oxalate are not affected; crystals of calcium carbonate dissolve with effervescence. If hydrochloric acid is applied to the section, crystals of calcium oxalate dissolve without effervescence; crystals of calcium carbonate dissolve with effervescence.

Polarized light affords a very delicate means of detecting the presence of crystalline or other earthy deposits in vegetable sections. Even if the crystals do not distinctly polarize, their forms, especially when quite transparent, are brought out in some positions of the Nicols.

Animal Specimens.

Many of the directions already given for the study of vegetable specimens might be repeated for animal specimens. The methods described for the manipulation of the object under the microscope apply equally well to the study of both classes of objects.

Animalcules. — No attempt will be made here to describe the microscopic forms of animal life. The subject is too extensive for cursory treatment. Some good manual should be consulted for their identification. Any of these forms may be examined under the microscope if placed in a drop of water on the slide, and a cover-glass laid over the specimen. The cover-glass should be supported by bits of other thin glass. A shallow cell made as described in the following chapter makes a good live-box for the temporary examination of these specimens. The specimens are transferred by means of a piece of glass tubing used as a pipette.

The stagnant water of swamps furnishes countless varieties of microscopic animal life. The more quiet the water, the more abundant the animalcules. Seek out small, isolated pools. Collect some of the water around submerged objects. In collecting, it is best to place some of the aquatic plants in the collecting vessel with the water, disturbing the plants as little as possible. The animalcules cling in large numbers to the plants. A jar of this swamp water will furnish instructive entertainment for many an hour.

Water Sediment. — Allow a considerable amount of water to stand in a tall glass vessel for several hours. With a piece of rubber tubing, siphon off all but a small remnant of the water. With a pipette, place a very small drop of

the sediment on a glass slip, and put a cover-glass over it. Examine with objectives of different powers, beginning with a low power. Solid particles of an earthy character will probably be found together with some microscopical forms of animal and plant life. The more impure the water, the more abundant these forms. The solid particles are probably either silica or the carbonate of lime. Run a little strong sulphuric acid under the cover-glass. Carbonate of lime is dissolved with effervescence of carbon dioxide, which will form numerous minute bubbles. Silica is unaffected by the sulphuric acid, and vegetable substances are more or less blackened.[1]

Striped Muscular Fibre. — The tissues of the larger animals may be prepared for examination by teasing them to pieces on the glass slip in a drop of water, glycerine, or some staining solution. The striated fibre of the voluntary muscles is thus easily prepared. Borax-carmine or hæmatoxylin is a good medium to employ. The fibrillæ, the nuclei, the transverse striations, and the sarcolemma, or investing membrane, are readily made out. The study should be begun with a low power, and continued with a high power.

A half per cent solution of common salt in water is also a good medium for this study. A preparation may be made by teasing up a bit of any of the active muscles of the legs of an animal in the salt solution. After the transverse striations have been studied, remove the salt solution and run acetic acid under the cover-glass. This causes the striations to show less distinctly, but the nuclei

[1] For more complete directions for the microscopical examination of water, see the author's "Water Analysis for Sanitary Purposes: Chemical and Biological." Boston, 1892. Willard Small, Publisher, 24 Franklin Street.

and the sarcolemma are brought more clearly into view. If now the acetic acid be washed out by applying successive drops of water to one side of the cover-glass and drawing them under by means of a piece of filter paper at the opposite side, and then a little borax-carmine stain be run in, the nuclei take a deep color and are rendered very conspicuous. They are somewhat elongated or fusiform, and seem to be at the very surface of the fibres.

Unstriped Muscular Fibre. — In a similar way a study may be made of the unstriped fibre of the involuntary muscles by teasing out a piece of the wall of the stomach or intestine. The elements of this muscle will be found to be elongated cells, each containing a nucleus situated near the centre, which stains more deeply than other portions.

Sections of Animal Tissues. — To obtain sections of soft animal tissues it is necessary to harden the specimen. This is most conveniently done by placing small pieces in alcohol for a few days. Better results are obtained if the specimen is gradually hardened by remaining a few hours in weak alcohol, say 50 per cent, then in 70 per cent and 95 per cent alcohol. The pieces should be not more than half an inch square and one-fourth of an inch thick, and a large volume of alcohol should be used. When sufficiently hard to give clean sections, the object may be held in the hand or between pieces of carrot or of pith moistened with alcohol, and sections cut by the free-hand method already described. Or the object may be freed from alcohol by soaking in turpentine a few hours, and imbedded in paraffin by the method described in Chapter IX. Both unstained and stained sections should be examined. The staining is best accomplished by allowing the sections to lie in a watch glass with a few drops of the staining fluid until the right intensity of color is

obtained. The best effects are secured when the specimen is treated, before and after staining, according to the directions given in the Appendix in connection with the formulas for the different staining solutions.

Blood. — Place on a cover-glass a small drop of fresh blood obtained by pricking a fold of the skin on the back of the wrist with a needle previously sterilized by passing it through the gas flame, and invert the cover-glass on a glass slip. Observe the numerous red corpuscles floating about in a colorless plasma. The corpuscles seem to attract one another, and are seen to move towards one another, forming rolls. The tendency to do this is increased if the cover-glass is slightly moved on the slip. The red corpuscles are seen to be round and very thin, regular in shape, disk-shaped, hollowing in the centre, or bi-concave. Among them the smaller and irregular white corpuscles may possibly be distinguished, but it is not always easy to do this, as in health the white corpuscles are very few in number compared with the red corpuscles. In some diseases the relative proportion of the white corpuscles is much increased.

Fresh blood from a frog is more favorable for the study of the white corpuscles. The white corpuscles are relatively much more numerous in the blood of the frog than in human blood. The form of the white corpuscle will be seen to be very irregular, the appearance being very much like the amœba. If the slide is slightly warmed, a true amœboid movement may be seen in these corpuscles, as the irregular projections change in form and size, moving the corpuscle about. The structure of the corpuscle is granular in the central portion; clear and transparent in the outer portions. If the specimen is treated with acetic acid and then with borax-carmine, the nucleus will stain,

showing one or more nucleoli; the outer portions remain unstained.

The red corpuscles of frog's blood are also very interesting. They are much larger than the corpuscles of human blood, oval in shape, and contain a distinct nucleus which is slightly convex, being thicker than the peripheral portions. If the specimen is treated with alcohol and then with hæmatoxylin or with borax-carmine, the nucleus takes a deep stain, the other portions remaining uncolored. Iodine is also a useful staining agent for blood corpuscles.

Very interesting preparations of frog's blood may be made as follows: Place a minute drop of the blood on a cover-glass and lay a second cover-glass over it. Rub the cover-glasses together to obtain a very thin evenly distributed film of the blood on each. Place the cover-glasses, prepared side up, on a sheet of paper and let them dry thoroughly in the air or exposed to direct sunlight. When dry, place on each a large drop of Kleinenberg's or Grenacher's hæmatoxylin and let it remain twenty minutes. Do not let it dry up. If there is a tendency to do so, add more of the stain. Wash thoroughly in water by letting a gentle stream fall upon the cover-glass, or, holding the cover-glass with the forceps, dip it in a considerable volume of water. When the excess of hæmatoxylin has been removed, place a weak solution of eosin on the cover-glasses and allow it to remain five minutes. Wash thoroughly again, and dry the cover-glasses as before. The nuclei are stained purplish blue; the peripheral portions red. Place a drop of Canada balsam on a clean glass slip and place one of the thoroughly dried cover-glasses on it, prepared side down. Press it down gently, and a very attractive and permanent object is made.

The double staining may also be accomplished by using successively different aniline stains; fuchsin and methylene blue, fuchsin and Bismarck brown, fuchsin and iodine green are recommended. In making the preparations, the cover-glass may be dried by passing it through the heated air several inches above a gas flame, holding the cover-glass with the forceps. Much care must be exercised, or the corpuscles will be shrivelled. It is better ordinarily to let the film dry at the ordinary temperature of the room.

The Circulation of the Blood in a Frog's Foot. — The interesting appearance of the blood circulating in the web of a living frog's foot may be seen at the cost of very little trouble. No special piece of apparatus is required, but a thin strip of wood five inches long and one and a half inches wide, with a hole three-fourths of an inch in diameter bored through it an inch from one end, makes an excellent frog plate. In the centre of a piece of cloth about eight inches square cut a small hole, and pass the frog's foot through it. Wrap the cloth around the frog and saturate it with water. With a soft string bind the frog loosely upon the strip of wood in such position that its foot comes over the opening. Tie threads to its toes, and so fasten them that a portion of the web is spread horizontally over the opening, and all is ready for examination. A little care must be exercised that the strings are not drawn too tight, or the circulation may be stopped. The frog does not appear to be hurt, and after the first fright is over usually lies very quietly.

Cartilage. — Cut as thin a section of cartilage as possible, and mount in a half per cent salt solution. Cartilage from the sternum of a frog gives particularly favorable specimens. Slowly run under the cover-glass some iodine solution. The cells of the cartilage will be brought into

clear view as they take a deep brown stain from the iodine. The cells occur singly, or two, three, or four together. When there are three, one is larger than the other two. The growth by cell division is evident from this arrangement of the cells. Each cell contains a nucleus; some contain two nuclei. The nuclei sometimes contain several nucleoli. Between the cells is a lifeless matrix which does not take the stain.

Bone. — Bone may be decalcified by treating it for several hours with very dilute hydrochloric acid. As the hydrochloric acid destroys some of the soft tissues, better results will be obtained if chromic acid is used, beginning with a one-sixth per cent solution. After twelve hours pour off this solution, and replace it with a one-third per cent solution, and after another twelve hours with a one-half per cent solution. Do not use a stronger solution than this, but use a large volume and allow the specimen to remain until softened. Wash thoroughly in water to remove the acid.

Sections may now be easily cut with a razor. Wash the sections in water to make sure that the acid is removed, and stain in borax-carmine, which imparts an intense color. Avoid overstaining, removing excess, if necessary, with acid alcohol.

It is not difficult to prepare sections of bone by grinding. A suitable piece of dry bone, a cross-section, for instance, cut as thin as possible with a surgeon's saw, is ground smooth on one side by rubbing it over a fine-grained whetstone or file. During the grinding, press gently but firmly upon it with the fingers. Now place a large drop of Canada balsam on a piece of glass about an inch square, and hold the glass with the forceps, or better with a spring clothes-pin, over the gas flame until the volatile portions

are driven off. Allow it to cool, but before it is quite ready to set, place the smooth side of the bone on it, and press it gently down until the balsam is cold. Using the glass as a holder, continue to grind the bone on the whetstone or file until it is thin enough. Wash the surface with alcohol, and examine the progress of the work from time to time under the microscope, placing a drop of water on the specimen and covering with a cover-glass. The section may be thus studied on the bit of glass, or it may be very easily made into a permanent mount. Immerse it in chloroform or benzole until the balsam dissolves and the specimen slips off from the piece of glass. Use no force to remove it, or it will be injured. Place a drop of balsam on a glass slip, and heat to drive off the volatile portions so that, upon cooling, the balsam becomes quite hard. Treat a cover-glass in the same way. Remove the section of bone from the chloroform or benzole, using a camel's-hair brush as a lifter, and drain off the fluid by touching the section with a piece of filter paper. Place the dried section on the centre of the slip, invert the cover-glass over it, and hold some distance above the flame until the balsam just melts. Set aside to harden. The object of this treatment is to prevent the balsam from penetrating the specimen and rendering the structure too transparent.

Epithelium.—Epithelium is to the internal cavities of the body what the skin is to the outside. It does not, however, form a firm and continuous covering to the underlying tissues. It exists in various forms of loosely joined cells. These forms run into one another insensibly, but are usually distinguished as pavement and columnar epithelium. The cells of the former are thin and flat. Their arrangement somewhat resembles the arrangement of the stones of a pavement. The cells of columnar

epithelium are thickly crowded together, arranged like aggregates of columns. The columnar epithelium may have cilia or hairlike appendages.

To obtain specimens for examination, scrape with a dull knife-blade the inside of the cheek, the roof of the mouth, or the mucous membrane of the intestine of a recently killed animal, and mount the scrapings in water, or a half per cent salt solution. The cells are nucleated. Scrapings from the tongue or the roof of the mouth of a recently killed frog are especially good for the study of ciliated epithelium. The movements of the cilia are best studied by watching the specimen under a high power for some time, while the cells die, the movements of the cilia in full life being too rapid to be observed well.

The methods of studying vegetable and animal specimens may, of course, be indefinitely extended. If sufficient has been said to give the beginner a start in the right direction and awaken interest in these fascinating processes of investigation, the object of this chapter is attained. The use of so many different stains and reagents is wont to seem to the beginner an almost insurmountable obstacle to the work. The expense involved is very slight, and a little experience shows that the trouble of the manipulative processes is small. As nearly all the reagents and stains keep well, and some are even improved by age, they should be prepared in leisure hours and kept ready for use in suitable bottles. When the trouble of using is thus reduced to a minimum, there soon comes to be a fascination in studying the effects produced by different reagents and combinations of reagents. A previous knowledge of chemistry is, of course, an advantage in this work, but is by no means a prerequisite.

CHAPTER VI.

MOUNTING OBJECTS FOR THE MICROSCOPE.

THE process of mounting objects for the microscope for permanent preservation is very simple. Many different media are employed, but it is well for the beginner to become thoroughly familiar with the working of one before attempting to use others. One who has mastered the methods given in this chapter will find no difficulty in using any medium.

Mounting in Canada Balsam. — The medium most universally employed is Canada balsam. This medium presents the fewest difficulties. It is therefore well suited to the needs of the beginner. Canada balsam may be obtained of dealers in microscopical supplies in the pure form, or combined with various solvents, as benzole, xylol, or chloroform. These solutions of balsam, when properly prepared, harden much more quickly than the pure balsam. In their preparation the pure balsam is heated at a moderate temperature until the volatile portions are driven off, so that when cold the balsam is hard and chips easily. This hardened balsam is then dissolved in benzole, xylol, or chloroform. When an object is mounted in this solution, the solvent quickly volatilizes, and leaves the cover-glass securely cemented to the glass slip by the hard balsam. Ignorant or unscrupulous dealers sometimes simply mix fresh balsam with the solvent, and sell the mixture under the name of benzole, xylol, or chloroform balsam. Such a mixture is as slow in drying as the pure balsam.

If the object to be mounted contains moisture, as is ordinarily the case, it must first be thoroughly dehydrated, or a cloudiness will result around the object from the fact that the water does not mix with the balsam. The dehydration may, in most cases, be sufficiently accomplished by soaking for some minutes in strong commercial alcohol, commonly spoken of as 95 per cent alcohol. The use of absolute alcohol will insure perfect dehydration, but there are comparatively few cases where it is a necessity.

After dehydration the alcohol is removed, and the specimen cleared, by placing it for a few minutes in oil of cloves, turpentine, benzole, or some other substance which drives out the alcohol, and which is itself miscible with balsam. Perfect clearing is important, and it is often well to pass the specimen through two changes of the clearing agent. Both dehydration and clearing may be effected after the object is placed on the slide. The specimen is covered with a large drop of alcohol or of the clearing agent. After a few minutes this is drained off, and another portion of the fluid applied. Repeat the process still again for especial thoroughness.

A glass slip is thoroughly cleaned and warmed over a gas flame to drive off all adhering moisture. The slip is placed on the turn-table, the object is exactly centred, and a small drop of Canada balsam is dropped upon it. The cover-glass, thoroughly cleaned and warmed, is allowed to fall gently upon this. The cover-glass should be handled with the forceps to prevent leaving finger marks or stains upon it. One edge should be brought in contact with the balsam at one side of the object. It should then be slowly and gradually lowered to position to prevent displacement of the object by a too violent movement of the balsam. The cover-glass should then be pressed down and exactly

centred. Gentle pressure may be applied, if desired, by means of spring clips; or by means of lead bullets slightly flattened by a blow from a hammer so that they will not roll; or by large-headed screws, which are excellent for this purpose.

The balsam may be allowed to harden at the ordinary temperature. If pure balsam is used, the hardening will require some weeks, so that it is often desirable to hasten the process. The slides may be placed in the oven of a water-bath, and a temperature of between 130° and 140° F. maintained for several hours. If a higher temperature is used, or if the heating is unduly prolonged, the balsam will turn yellow. Another way is to put the slides in the kitchen oven at night just after the fire is dumped. In the morning the balsam will ordinarily be well set. A steam radiator or the mantle-piece above a stove serves a similar purpose. The slides should of course be protected from dust. Each should bear a temporary label to prevent mistakes in identification.

This is all that is really necessary for a permanent mount. The object will be preserved indefinitely without further treatment. With practice it is not difficult to gauge the amount of balsam so that the space between the cover-glass and the glass slip will be exactly filled without overflow. If this has not been accomplished, and there is an overflow of balsam, the slide may be placed on the turn-table, and the thoroughly hardened balsam cut off with a chisel-shaped instrument. A little water placed on the slide will prevent the balsam chips from flying about, and will help in cleaning the slide. When the superfluous balsam has been dislodged by means of the chisel, the slide is placed under a gentle stream of water from a faucet and carefully rubbed with a small brush. The final portions

may be removed with a piece of cloth wet with alcohol, after which the slide is carefully wiped with a dry cloth.

Mounting in Glycerine-Jelly. — To mount with glycerine-jelly requires rather more skill than to mount with Canada balsam, on account of the difficulty of getting rid of air bubbles, which must be removed before the mount is sealed. There are, however, no serious obstacles to be overcome. For many objects, noticeably delicate plant sections, glycerine-jelly is superior to balsam. Delicate tissues are shrivelled by dehydration. Glycerine-jelly does not require that the water should be removed from the specimen, as water and the jelly are readily miscible.

The bottle of glycerine-jelly is placed in a dish of water, and the water heated until the jelly melts. Carefully clean a slide, place it on the turn-table, put the object on its centre, and breathe upon it. Working quickly, place a drop of the melted jelly on the object, removing any air bubbles by pricking them with a needle. Breathe upon a clean cover-glass, put a little of the hot jelly upon it, invert it over the specimen, and centre exactly. If air bubbles are formed under the cover-glass, they may sometimes be removed by introducing a fine wire.

Another method of procedure is to place a small piece of the solid glycerine-jelly on the centre of the glass slip, and carefully warm until it melts. The object is placed in this, and the air bubbles are removed, if any are formed. A piece of the glycerine-jelly is placed on a cover-glass held in the forceps, and melted. The cover-glass is then inverted over the object and centred.

Set the slide away with a light weight upon it for twenty-four hours for the jelly to harden thoroughly. Remove the superfluous jelly with a knife and by washing

in *cold* water, using a cloth wet with water to remove the final portions.

Glycerine-jelly mounts must be sealed with some tough cement to prevent the entrance of air and the parting of the cover-glass from the slide. Preliminary to doing this the glycerine-jelly around the edge of the cover-glass may be firmly set and rendered insoluble by slightly moistening it with a strong solution of potassium dichromate and exposing to the sunlight. The slide is placed upon the turn-table, and the potassium dichromate applied with a brush. When the slide is ready for the cement, a sable-hair brush with long hair is lightly charged with some transparent cement, such as shellac cement, or Berry's hard oil finish (see Appendix). The turn-table is rapidly whirled and a light ring of cement formed about the edge of the cover-glass. The brush is charged a second time, and the ring is reinforced by a second coat. The slide is now set aside for the cement to harden. Several additional coats should be applied at intervals until a firm ring has been formed to hold the cover-glass securely in place.

Mounting in Thin Fluids. — It is sometimes desirable to mount in clear glycerine, or in some fluid of even less consistency. The principal difficulty arises from the ease with which the cover-glass is displaced when it is attempted to apply the cement ring. To overcome this difficulty, after the cover-glass is in position over the object, and the superfluous fluid which runs out from under the cover has been removed with a piece of filter or blotting paper, place minute drops of melted paraffin or sealing wax at three equidistant points around the cover-glass. Upon hardening, these drops will hold the cover-glass in position while the rings of colorless cement are being applied. In many cases a shallow cell, such as is described in the next para-

graph, is required to hold the fluid medium, especially when the object is of appreciable thickness.

Dry Mounting. — Some objects are rendered too transparent when mounted in any of the above-mentioned media. The markings on starches, scales of moths, diatoms, and many similar objects are much less distinct when imbedded in these media than when they are viewed dry. The principal objection to dry mounting is the fact that after some time moisture is liable to collect in the mount and obscure the view of the object. For dry mounts glass slips should be prepared with shallow cells, formed of rings of transparent cement, of a size to suit the cover-glass to be used. These cells should be well seasoned before they are used. Many of the slips may be prepared in leisure hours and kept on hand ready for use.

When ready to mount an object, run a *very thin* fresh coat of transparent cement on the outer edge of the hardened ring. Place the object in position on the slip, or, if light like starch or the scales of a moth, dust a little of it on a cover-glass. Lay the cover-glass, prepared side down, in position over the cement ring. The ring of fresh cement should be of such size that the edge of the cover-glass will extend into it slightly all around. Place a light weight on the cover-glass and set the slide away until the cement is hard. Then place it upon the turntable again and apply a *light* coat of cement over the juncture of the cover-glass with the cement ring. If too much cement is used, it will surely run under the cover-glass. When the first coat is dry, add another light one, and repeat until the mount is thoroughly sealed.

The sweating of dry mounts may in a measure be prevented, when the object will not be injured by exposure to the air, by making small openings through the hardened

cement with a fine needle to give free circulation of the air through the cell. Much care must be exercised in making the opening, or the cover-glass will be broken.

Cells of paraffin are also much used for dry mounts. They are very easily prepared. The paraffin is heated quite hot and applied with a brush in the usual way of forming rings on the turn-table. The ring hardens in a few minutes and is then neatly trimmed down with a chisel-shaped instrument, using the turn-table as a lathe. A coat of transparent cement is applied to the ring, and the object is mounted and sealed as already described.

Mounting in Cells with Fluid Media. — It is often desired to preserve objects that are too thick to mount without some means of raising the cover-glass from the slide. Many delicate objects — whole insects, for example — would be crushed and much injured if mounted in the ordinary way, unless especially prepared for that style of mounting. All such objects may be mounted in cells of depths suited to each case. When the objects are quite thin, rings of transparent cement of sufficient thickness may be built up. These should be prepared beforehand and kept ready for use. A well-seasoned cell is much more reliable than one freshly made. In making these cells apply light coats of the cement at intervals, giving time for each to dry before the next application is made. For thicker objects, rings of glass, hard rubber, or metal should be purchased of some dealer in microscopical supplies. These are fastened to glass slips by smearing one side with transparent cement, placing them in exact central position on the slip on the turn-table, and setting aside with a light weight on them until the cement is hard. A coat of the cement is then run around the outside of the cell and allowed to harden.

In mounting, the cell is filled even full with Canada

balsam, glycerine-jelly, glycerine, or other mounting medium to be used, taking care not to let the medium touch the upper surface of the ring. Place the slide on the turn-table and run a ring of transparent cement on the upper surface of the ring. Lower the cover-glass to position and see that there are no air bubbles. The edge of the cover-glass should be slightly imbedded in the ring of fresh cement. If skilfully done, the cement and atmospheric pressure will hold the cover-glass in position. Any overflow of the mounting medium should now be removed unless the medium is Canada balsam. It is generally better to wait for the balsam to harden before attempting to remove it. Aqueous media may be washed away, the cover-glass being held in position with spring clips, if necessary. After this cleaning, the cover-glass is secured in position by successive light rings of transparent cement.

It is important for success in cell-mounting that the upper surface of the cell be perfectly even and flat. Cement cells may be brought to this condition, when necessary, by means of a fine file, or by heating to soften the cement, and pressing squarely against the top of the cell with a glass slip. No attempt should be made to use curtain rings and other cheap substitutes for cells, as no amount of skill will enable one to succeed in closing the cell if its upper surface does not make perfect contact with the cover-glass all around.

Finishing Slides. — For practical purposes, slides made by the processes above described may be regarded as finished. The appearance and the durability of the slide will, however, be much improved by applying neat rings of colored cements. Before applying these colored cements, balsam as well as other mounts should be thoroughly

sealed by the application of several light coats of transparent cement. When this cement has thoroughly hardened, the edges should be turned true with a chisel-shaped instrument, using the turn-table as a lathe. A somewhat broad ring of some colored cement may now be applied, entirely covering the transparent cement and extending slightly on to the cover-glass on the inner side and out on to the slip on the outer side. Give this cement time to harden and turn both edges true. A narrower ring of some contrasting color is now applied in such a manner as to leave a narrow ring of the first color on either side. When dry, a ring of a third color may similarly be applied so as to leave a ring of the second color on either side of it. With a little practice, rings of as many different colors as desired may be very quickly and neatly applied, and the appearance of the slide much improved.

There is in some quarters a professed contempt for this ornamentation of slides. Carried to excess it is unquestionably a serious waste of time. The contempt usually comes from those who have never acquired the skill to do a neat piece of work. One who has once acquired this skill knows that he possesses the ability to do neat work more rapidly than he could before do slovenly work. There is, too, a real pleasure in a piece of neatly executed mechanical work, and skill attained in this work is a training for the many other mechanical operations of the microscopist.

In applying cement rings, a long-haired sable brush should be used. The brush known among artists as the Rigger brush is excellent. The brush should first be lightly charged and the turn-table rapidly whirled. This first application forms a track which the cement will follow in the subsequent applications. The brush is now more

heavily charged so that a *small* drop forms at the end. The turn-table is rapidly whirled, the brush held nearly vertically or slanted in a line tangent to the ring to be formed, and the drop of cement approached until it just touches the track formed for it. The judgment must be used as to the amount of cement to be now applied. As a rule it is better to apply the cement in light coats, giving time for each to harden. In this way the spreading of the cement is avoided.

It now remains to place neat permanent labels on the slide, and it is finished. These labels should state the common and scientific name of the object, call attention to any particular points of structure or preparation, name the mounting medium and stains employed, the thickness of the cover-glass, and any other facts which will be of value in future examinations of the slide.

Cleaning Glass Slips and Cover-Glasses. — It often happens even with experienced microscopists that imperfect mounts are made, and an easy way of cleaning the slip and cover-glass is desirable. A wide-mouthed bottle should be provided for this purpose, a pint fruit jar, for instance. In this place a strong solution of borax, washing soda, or caustic potash. Into this solution drop the spoiled slides, here to remain until a number have been so deposited, and until a convenient season presents itself to attend to them. The solution dissolves not only balsam, but all resinous media and cements. After a few days the cover readily slips off from even seasoned mounts, and the balsam and cements are easily removed by scraping with the thumb nail. Sharp instruments should not be employed to scrape a slide that is to be used again, as scratches are almost sure to result.

The writer uses ordinarily borax water, as being most

agreeable to handle, and finds the glass clean enough for most practical purposes. To make sure that the slides and covers are optically clean, soak them for some days in the following mixture: dissolve 10 grammes of potassium dichromate in 50 c.c. of water by the aid of heat. When the solution is cool, immerse the containing vessel in cold water, and pour into it 50 c.c. of sulphuric acid slowly and carefully with constant stirring.

Upon removing the slides and cover-glasses from this solution, wash them thoroughly in clean water.

CHAPTER VII.

PRACTICE MOUNTING.

The object of this chapter is to furnish illustrations of the methods of the preceding chapter, and to afford means of introducing the beginner to some of the manipulations necessary in the making of microscope slides. An object prepared for the microscope is not unfrequently an object of supreme mystery to one who is wholly inexperienced in the processes. Minute and explicit directions will be here given for a few simple, easily accessible, and easily prepared objects. If the beginner will follow these directions with patience and care, the mystery will be solved and be proved to be no mystery at all.

For this practice work the necessary equipment consists of a few glass slips and cover-glasses; a small bottle of benzole or xylol balsam; a bottle of sodium or calcium hypochlorite; a bottle of borax-carmine staining fluid; one each of iodine green, alcohol, turpentine, benzole, and a weak solution of caustic potash; a pair of improvised dissecting-needles; and plain forceps. With this equipment, not only the work of this chapter, but quite a wide range of investigation may be accomplished, and a considerable variety of work in the preparation and mounting of objects may be done. It is better, perhaps, to purchase the borax-carmine staining solution ready for use, though it is not difficult to prepare it according to the directions given in the Appendix. However it is obtained, it is of importance to know its alcoholic strength, and to soak the object to be

stained in alcohol of the same strength before it is placed in the stain, and after staining to rinse off the the superfluous staining fluid in alcohol of the same strength. If these precautions are neglected, solid particles of carmine will be deposited on the specimen. The iodine green should be purchased in the solid form. A strong solution of this in alcohol should be made and filtered. This solution may be diluted with alcohol to any desired degree for use.

Insect Scales, Starch, and Similar Light Objects. — These objects afford interesting material for slides, and are especially easy of preparation. The wing of a moth is touched to a cover-glass, or a little starch is dusted on a cover-glass, and the cover-glass is inverted over a small drop of balsam on a glass slip. Hold the clean cover-glass in the forceps by one edge, bring the opposite edge into light contact with the balsam, and allow the cover to fall by its own weight gradually upon the balsam. Carefully press down and exactly centre the cover-glass. Pay no attention to air bubbles unless they are quite large, as they will disappear as the balsam hardens. The centring of the object and of the cover-glass may be accomplished on the turn-table, or by using the common expedient of drawing a figure one by three inches in dimensions on a slip of paper, finding its centre by drawing diagonals, and describing a circle the size of the cover-glass about this centre. The balsam is now hardened, and the slide cleaned and finished according to the directions given in the preceding chapter.

Hairs. — Hairs should be soaked in turpentine for some days to render them more transparent. They should then be cut in lengths suited to the size of cover-glass used, placed on a glass slip, a small drop of Canada balsam

allowed to fall on them, and a clean cover-glass placed over them. Many hairs polarize finely. Among easily accessible and interesting hairs may be mentioned human hairs (especially those of the beard), caterpillar, rat, mouse, raccoon, and bat hairs, and the bristles of the hog. The hog's bristles are especially attractive objects under the polariscope.

Insects. — When the beginner has acquired a little experience in mounting the above-mentioned objects, he may advance to parts of insects, as the wing or foot of a fly, and to the mounting of small insects whole. Soak the object for twelve hours in a weak solution of caustic potash to cleanse it from any adhering dirt or oily particles, and to soften the harder portions of chitinous skeletons. If a whole insect is being treated, it should now be placed between two pieces of glass, and the soft visceral portions removed by gently and carefully pressing the pieces of glass together. The soft portions are then carefully brushed off, placing the piece of glass bearing the insect under water in a shallow dish and using two camel's-hair brushes, one to hold the insect, the other to remove the adhering matter. Renew the water until the caustic potash has been wholly washed out. Place the object in 95 per cent alcohol for twenty-four hours to remove the water. Then treat with turpentine. The longer the object remains in the turpentine, the clearer and more transparent will it become; but as the balsam has a similar effect, it may remain in the turpentine only long enough to become permeated. It is well to allow it to remain at least twenty-four hours. Warm a glass slip by passing it to and fro above a gas or lamp flame to drive off all moisture. Transfer the object to the centre of the slip and drain off the superfluous turpentine. Place a drop of Canada

balsam on the object and cover with a previously warmed and cleaned cover-glass.

The insect world offers such variety that one might spend his life in studying and mounting insect preparations. A dissecting-microscope is of great use in separating and dissecting out by means of dissecting-needles the parts to be mounted. Parts which are too large to be mounted as above must, of course, be mounted in a cell prepared as described in the preceding chapter. The process of mounting is similar and but little more difficult than when no cell is used, as long as balsam is the medium employed.

Vegetable Sections. — Place some transverse sections of a plant stem, — a geranium stem is good for the first trial, — cut by the free-hand method described in Chapter V., in a considerable volume of sodium hypochlorite for several hours to bleach them. Wash thoroughly in several changes of water and transfer to 35 per cent alcohol. In a few minutes transfer to borax-carmine stain. Let the sections remain in the stain until upon the examination of one under the microscope it is found to be sufficiently colored. This operation will require a length of time varying from a few minutes to several hours, depending on the strength of the fluid. Transfer to slightly acid 35 per cent alcohol. In a few minutes transfer to a weak solution of iodine green in alcohol, where the sections should remain at least half an hour. They will probably now appear of a uniform green color. Place in 95 per cent alcohol until the green is removed from the parenchymal portions, but take care that they do not remain too long, or the green stain will be wholly discharged. Transfer to oil of cedar or to benzole to remove the alcohol and clear. After a few minutes mount in balsam.

Sections which will bear repeated transferals are best

treated in watch glasses or individual butter dishes of white porcelain. When the sections must be left for some time in a fluid, two-drachm vials, to be obtained at the drug store, are very convenient, as they may be set away for hours tightly stoppered, and there is no loss by evaporation, as would be the case if watch glasses are used.

Preparations made by teasing, and any delicate tissues, may be passed through these processes on the glass slip. In each operation cover the specimen well with a large drop of the fluid. It is well to repeat the washing in alcohol and the clearing in oil of cedar or benzole to make sure of thorough work.

Animal Sections. — Place some sections of animal tissues, cut as described in Chapter V., in 70 per cent alcohol for a few minutes, transfer to Kleinenberg's hæmatoxylin, letting them remain until, upon the examination of one under the microscope, they are seen to be sufficiently colored. Remove to 70 per cent alcohol, and in a few minutes transfer to 95 per cent alcohol, then to absolute alcohol, and to oil of cloves. Mount in Canada balsam.

If it is desired to use other stains, follow the directions given in the Appendix in connection with the formula for each stain for the treatment before and after staining. Use a camel's-hair brush to handle the sections. Both animal and vegetable sections may be secured on the centre of the glass slip by means of white of egg or Schällibaum's collodion fixative, used as described in the Appendix.

Diatoms. — It is not the purpose here to go far into the very extensive subject of diatoms. The attention of the beginner is more particularly called to recent diatoms. At some pond, stream, or spring, gather some of the brown or yellowish brown slime adhering to any of the

water plants. Place some of this slime in a test-tube with enough nitric acid to cover it, and boil for some minutes. Add a small quantity of potassium dichromate and boil again. The diatoms will usually be well cleaned by this process, if it is continued sufficiently long. Pour the contents of the tube into a large volume of water in a tall glass vessel and allow the vessel to stand undisturbed for several hours until the diatoms have settled. Without disturbing the diatoms at the bottom, decant off the liquid above them. Fill the vessel with clean water and allow it to stand again. Repeat this process until the acid has been entirely removed.

If, now, it is desired to separate diatoms of different densities, fill the vessel again with water, and let it stand until the heavier diatoms have settled. Decant off the water containing the lightest diatoms. Fill the original vessel again with water, and again let the heavier forms settle. Decant the lighter forms into another vessel. By a continuance of this process the diatoms of different weights are quite easily separated. The sediments from the different vessels used are finally collected in small bottles with a little water or alcohol.

To mount the diatoms, shake the bottle, place by means of a pipette a drop of the water containing the diatoms on a cover-glass, evaporate to dryness by holding the cover-glass above a flame, moisten with turpentine, place a small drop of balsam on the cover-glass, heat until the balsam boils, invert over a glass slip, centre, harden, and finish, as already described.

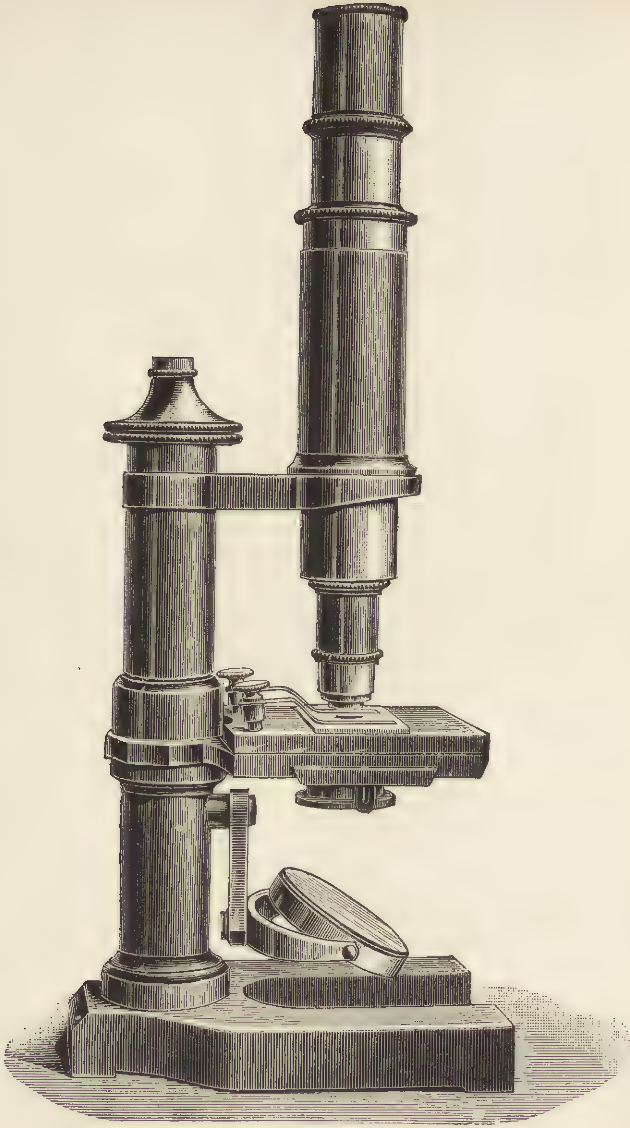
If one has the desire and patience to do the, as it seems to the writer, unprofitable work of arranging diatoms in figures, coat a cover-glass with gelatine fixative and let it dry. Under a low power arrange the diatoms in the

desired pattern, using a mechanical finger, or simply a hog's bristle, or cat's whisker, attached to a wooden handle by means of wax. When the pattern is formed, or from time to time during its formation, breathe gently on the cover-glass to soften the gelatine. Upon drying, the diatoms will be held fast in the gelatine. Mount in balsam.

Some of the fossil diatoms may be cleaned by the process described above. Others it is more difficult to clean. Much of the foreign matter may in some cases be removed by heating the earth to a red heat, placing it for this process on a piece of platinum foil or of porcelain. It is frequently necessary to break down the mass of diatomaceous earth by boiling with a solution of caustic potash or caustic soda. These solutions attack the diatoms, forming silicates of potassium and sodium. If they are used, the action should not be allowed to continue long, but as portions of the mass are broken down, they should be poured into a large volume of water to wash out the reagent. It is, perhaps, better to break down the mass of earth with ammonia of moderate strength, as ammonia does not act upon the diatoms to any perceptible extent. When the mass has been broken down, pour the diatoms into a considerable volume of water, and allow them to settle. This requires several hours. Decant off the water, and place the residue in a dish in which it may be heated. If the foreign matter cannot now be removed by the use of nitric acid with potassium dichromate, use hydrochloric acid, aqua regia, and sulphuric acid successively.

Certain earths are best treated, after the breaking down with ammonia, by dropping in powdered potassium dichromate while the residue is boiling, and then pouring in strong sulphuric acid slowly and with constant stirring.

Some workers recommend boiling the diatoms in strong sulphuric acid, at the same time sifting in pulverized potassium chlorate. This process is dangerous, as violent explosions are liable to occur. It should be a last resort. Flocculent matter is often very troublesome. It may sometimes be removed by boiling the diatoms in a strong solution of soap. The processes are long and tedious. Fine sand can be removed only by the process of decantation already described for separating the diatoms of different weights. Gatherings of the thin coating of black mud on the bottoms of springs, ponds, and streams may be treated in this way and yield rich results in fresh diatoms.



Leitz's Stand III.
Franklin Educational Co., Agents, Boston.
Fig. 34.

CHAPTER VIII.

BOTANICAL SECTIONS.

In some of the preceding pages a few simple directions have been given for the preparation and mounting of vegetable specimens. If a considerable study of structural botany is to be made, more elaborate methods leading to more perfect results are desirable. Even if one is not especially a student of botany, the making of plant sections is attractive; from no other material can showy and interesting specimens be so easily prepared as from plant life.

For purposes of sectioning, vegetable specimens may be divided into two classes.

1. Hard specimens, or those that cannot be cut without softening.
2. Soft specimens, including annuals, young shoots, buds, seeds, cones, and similar products of plant life, many of which must be hardened or otherwise prepared by special processes.

Hard Specimens. — Most solid wood specimens may be sufficiently softened for cutting by soaking in water for some hours, changing the water occasionally, or by boiling in water for a few minutes. Three pieces of each specimen should be prepared to give sections in three different directions, — transverse, radial, and tangential. A transverse section is made across the stem in a direction transverse to the length. A radial section is made lengthwise the stem,

along a radius of the circles of annual growth, or from the bark towards the centre of the tree. A tangential section is made lengthwise the stem, tangent to the circles of annual growth.

If no microtome is available, the pieces should be two or three inches long, that they may be firmly held in the hand, and about one-fourth inch in cross-section. Use a razor ground flat on one side, and follow the directions for cutting free-hand given in Chapter V.

For the microtome, specimens should be cut about one-fourth inch square and one inch long. If a microtome having a clamp is used, the softened piece of wood is fixed in the clamp and cut without further preparation. The nearer to the supporting clamp the section is cut, the more perfect it will be, as the springing of the specimen is reduced to the minimum. If a well-microtome is used, the specimen is fixed in the well by wedges of pine or other very soft wood which are sunk in the well, and do not come within one-fourth of an inch of the cutting plate. These wedges hold the specimen firmly, but leave one-fourth of an inch of it exposed for cutting. The specimen is placed at one side, close to the wall of the well, so that the wall may give it support and prevent the specimen from springing. In cutting, keep the knife and the specimen wet with water, dilute glycerine, or a mixture of glycerine and alcohol, using a camel's-hair brush to apply the liquid. Transfer the sections from the knife to a glass of water with a brush, or by dipping the blade of the knife in the water to float the section off.

Soft Specimens. — All specimens of a consistency suitable to be cut without special preparation may, of course, be cut free-hand, or may be placed directly in the clamp of the microtome and be cut at once. In many cases, how-

ever, immersion in alcohol for a few days is an advantage, when the changes resulting from the alcohol are of no account, as the alcohol removes all resinous substances, which stain and clog the knife. If the specimen is made hard and brittle by the alcohol, immerse it for a short time in a mixture of alcohol and glycerine, half and half, which will restore it to a soft condition.

Specimens which are too soft to be cut directly may be held between two pieces of cork, the specimen projecting about one-fourth of an inch above the cork. The whole should be clamped low down in the jaws of the microtome clamp to prevent springing. Do not cut through the cork, as it dulls the knife. Some specimens are advantageously held between pieces of pith, carrot, potato, or similar substance which will not injure the knife. Others are so soft that they must be imbedded in paraffin or celloidin. Some even require to be hardened before they are imbedded. The hardening is accomplished by gradual dehydration to prevent shrinkage. The specimen is placed in 50 per cent, 70 per cent, and 90 per cent alcohol, remaining in each several hours. Soft tissues thus hardened may be kept indefinitely in 90 per cent alcohol. So also tissues which do not require hardening may be preserved. Specimens collected in July or August may be thus kept for winter use. They are very conveniently kept in two-drachm vials. The specimen should be completely covered with the alcohol, and each vial should be labelled. The more woody specimens, which become hard and brittle in the alcohol, must be soaked twenty-four hours in the mixture of alcohol and glycerine before they are cut. In cutting alcoholic material, keep the specimen and knife wet with dilute alcohol. Handle the sections with a brush.

Imbedding in Paraffin. — Three grades of paraffin may

be obtained of dealers in microscopical supplies: hard, melting at 130° F.; medium, melting at 125° F.; soft, melting at 120° F. They may be mixed in any proportions necessary to give the right consistency for the temperature of the room in which the sectioning is to be done. Paraffin that will melt at about 125° F. is suitable in most cases. For paraffin moulds, obtain at the gas-fitter's pieces of the brass tubing used as the casing for gas fixtures. These pieces should be about one inch long and from one to two inches in diameter.

Prepare the object by dehydrating in alcohol of gradually increasing strength, up to absolute alcohol. Soak in chloroform twenty-four hours, then twenty-four hours in chloroform in which has been dissolved as much paraffin as will dissolve. As chloroform is somewhat expensive, the spirits or oil of turpentine is often substituted for it.

Melt the paraffin over a water-bath, but be sure that it is no warmer than is necessary to keep it fluid, or the specimens may be shrivelled. Pour on to a piece of paper enough of the melted paraffin to make a circle a little larger than the mould to be used. As the paraffin begins to stiffen fix the specimen upright in it, the end to be cut being down. Place a mould over the specimen, sinking it down into the circle of paraffin, and fill it with melted paraffin. Set aside, for the paraffin to become thoroughly hard. The hardening may be hastened, if so desired, by placing the whole in cold water as soon as the paraffin has stiffened sufficiently to bear this treatment. When the paraffin is hard, pass the mould two or three times through a gas flame, and the paraffin cast may then be easily pushed out. Trim off the opposite sides so that it may be more easily held in the jaws of the microtome, and cut away the paraffin to within an eighth of an inch of

the object on the end to be cut, and it is ready for sectioning. The cast may be held directly in the clamp of the microtome, or it may be fastened to the roughened end of a cork by means of a little melted paraffin, and the cork held in the jaws of the clamp. If a well-microtome is used, the object may be imbedded in paraffin in the well. Imbedded specimens may, of course, be cut free-hand in the manner already described. Keep the specimen and the knife well wet with alcohol, applying it with a brush. Transfer the sections with a small brush to chloroform, turpentine, benzole, or oil of cloves, any one of which will dissolve the paraffin. Remove this solvent by transferring to two changes of alcohol before staining.

Imbedding in Celloidin. — Celloidin may be obtained of dealers in microscopical supplies. It is a form of nitrocellulose. It dissolves in a mixture of equal parts of ether and absolute alcohol. Prepare two solutions, one quite thin; the other, thick. The object, which for this method should be not more than one-third of an inch in length, is thoroughly dehydrated in absolute alcohol and soaked in the thin solution of celloidin for twenty-four hours or longer. It is then transferred to the thick solution, where it is to remain some hours. It may then be imbedded as follows: Place a little of the thick solution of celloidin on a cork and let it dry for a few minutes. Add successive layers of celloidin, letting each dry, until a considerable mass is built up. Transfer the object from the thick solution of celloidin to this mass and build the celloidin up over it. Weight the cork and immerse the whole in 82 per cent alcohol, and in twenty-four to forty-eight hours the celloidin will be sufficiently hardened to cut. Stronger alcohol dissolves the celloidin and must not be used. Objects imbedded in celloidin may be kept

indefinitely in 82 per cent alcohol. In cutting, keep the object and the knife wet with 50 to 70 per cent alcohol. Plant sections should be cut to the thickness of one row of cells, which varies in different specimens from $\frac{1}{400}$ to $\frac{1}{1200}$ of an inch.

It is to be understood that the somewhat complicated processes of imbedding in paraffin and celloidin are not recommended for general use. The simple processes already described of cutting the sections without imbedding the specimen, or after imbedding it in pith or carrot, are amply sufficient for the greater number of objects that are to be cut, and will naturally be used, on account of their greater simplicity, when good sections can be obtained by them. There are, however, many delicate tissues that cannot be cut into thin sections unless their parts are supported by some substance that will infiltrate into the specimen. Resort is then had to the more troublesome methods. Another method highly recommended for this class of objects by many experienced workers is

Imbedding in Transparent Soap. — The soap mass is prepared as follows: Any good white soap is cut into thin slices and thoroughly dried by exposure to the sun. The soap is then crumbled to a fine powder and mixed with 90 per cent alcohol to form a stiff mass. Glycerine and 90 per cent alcohol are then added till the proportions by weight are: soap, 10 parts; glycerine, 22 parts; alcohol, 35 parts.

Place the object in 90 per cent alcohol, imbed in the soap liquefied by heat, cut sections dry or wet with alcohol, and remove the soap with warm water or alcohol.

Staining. — The staining of vegetable sections is a very simple matter. Many sections take the stain more readily if they are first bleached. Sections cut without imbed-

ding, or after being freed from paraffin, are placed in water and then in an aqueous solution of calcium or sodium hypochlorite in a stoppered bottle. The time required for bleaching varies with different specimens from one or two hours to one or two days. The specimens must be watched to guard against maceration. If the action is slow, decant the calcium hypochlorite and add a fresh portion from time to time. The bleaching will be more complete if the sections are transferred from the calcium hypochlorite to very dilute hydrochloric acid for a few minutes. After the acid wash thoroughly in water to remove the acid. The sections may now be stained in carmine, hæmatoxylin, or in aniline dyes. The Appendix gives formulas for preparing the staining solutions, but the beginner is advised to obtain the stains ready prepared by some dealer in microscopical supplies. After some experience has been acquired with the ready-prepared stains, trials may be made in preparing one's own stains. It is best to confine one's self to a very few kinds until some experience has been gained. As a rule alcoholic stains are more satisfactory than the aqueous stains, if the object is to be mounted in balsam. In using the carmine stains it is to be remembered that it is very important that the object be immersed in alcohol of the same strength as the stain before staining, and transferred from the stain to alcohol of the same strength. If this precaution is not observed, solid particles of carmine will be precipitated in the cells of the tissue. The object is then passed through the higher grades of alcohol to complete dehydration.

Objects cut in celloidin may be transferred to water or dilute alcohol and stained in carmine or hæmatoxylin. The celloidin is not colored by these stains, and the sec-

tions are mounted without removing the celloidin. Aniline dyes stain the celloidin, and must not be used for these sections. After staining transfer to alcohol of the same strength as the stain, then to water, then to glycerine, in which the sections should soak some minutes before mounting. Mount in glycerine or glycerine-jelly. If Canada balsam is to be the mounting medium, transfer the section from the stain to alcohol of the same strength as the stain, dehydrate in 95 per cent alcohol very quickly, clear with chloroform, oil of cedar, oil of bergamot, or oil of origanum, but *not* with oil of cloves, which dissolves celloidin.

It is to be remembered that the object of staining is to differentiate structure, and that that staining is most successful which brings out most perfectly the different parts of the specimen. Overstaining obscures structure and defeats the very purpose for which the staining is undertaken. Delicate staining also, as a rule, gives results that are in better taste than deeply stained objects. The thicker the section, the more careful must one be not to overstain. It is also to be remembered that many specimens are not suitable for double staining. The more homogeneous the structure, the less likely is the specimen to take two different stains.

The same dye prepared by different formulas acts differently on the same tissues. Experience alone can tell which stain, or which combinations of stains, will give the most satisfactory results. The same staining fluid also acts quite differently on sections of different plants. The acidity, alkalinity, or other chemical properties of the plant tissue modify the effect of the stains, so that each plant is, as it were, a law to itself in its behavior towards the staining fluid.

Order of Procedure with Vegetable Specimens to be cut in Paraffin.

1. *a.* If the material is fresh, dehydrate in 50 per cent, 70 per cent, 95 per cent, and absolute alcohol.
   b. If the material is alcoholic and hard, soak in alcohol and glycerine, equal parts, until soft; if the material is alcoholic and soft, omit this step.
2. *a.* Remove alcohol by soaking in benzole or oil of cloves. Soak 24 hours in benzole containing all the paraffin it will dissolve. Or,
   b. Soak in chloroform 24 hours, then 24 hours in chloroform in which has been dissolved as much paraffin as will dissolve.
3. Imbed in paraffin.
4. Cut sections.
5. Remove paraffin by soaking in chloroform, turpentine, benzole, or oil of cloves.
6. Remove chloroform, turpentine, benzole, or oil of cloves by soaking in two changes of 95 per cent alcohol. Bleach the section, if it seems desirable.
7. *a.* Place in 35 per cent alcohol.
   b. Stain in Grenacher's Borax-Carmine.
   c. Place in 35 per cent alcohol, to which has been added a drop or two of hydrochloric acid.
   d. Pass through 50 per cent, 70 per cent, and 95 per cent alcohol.
8. *a.* Stain in an alcoholic solution of iodine green or methyl green.
   b. Wash out excess of stain in 95 per cent alcohol, and place for a few minutes in absolute alcohol.
9. Clear in oil of cedar or benzole.
10. Mount in pure Canada balsam.

Number 8 may be omitted. In this case, place the sections in absolute alcohol, clear in oil of cedar, oil of cloves, oil of marjoram, oil of turpentine, or benzole. Mount in pure balsam or in balsam dissolved in xylol, benzole, or chloroform. Sections stained in the aniline dyes are liable to fade in oil of cloves and in xylol, chloroform, or benzole balsam, or in glycerine, or glycerine-jelly. They should,

therefore, be cleared in benzole or oil of cedar and mounted in pure balsam. Even then the aniline dyes are not very reliable for permanent mounts. Sections stained with these dyes should be kept in the dark, as exposure to strong light fades the colors.

If the sections are to be mounted in glycerine or glycerine-jelly, the above order may be adhered to through the staining. The sections pass from the stain into alcohol of the same strength as the stain, then into water. They are then soaked some time in glycerine, and mounted in glycerine or glycerine-jelly.

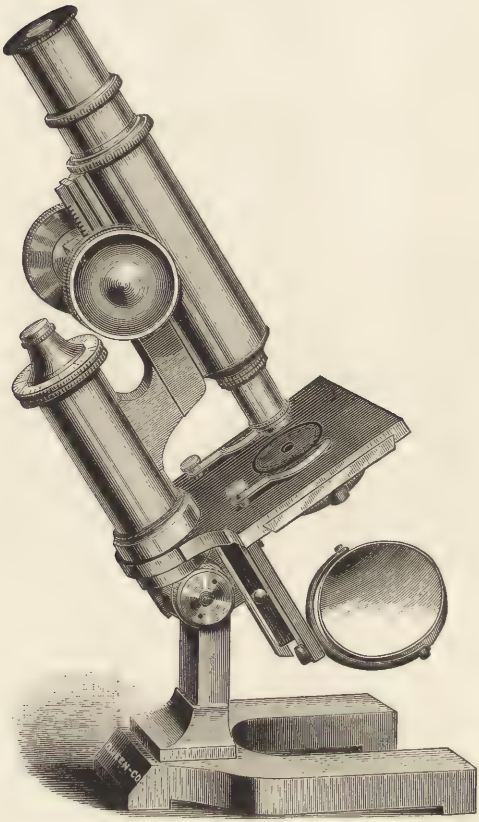
In the place of numbers 7 and 8 may be substituted any of the stains mentioned in the Appendix, taking care to treat the specimen before and after the staining according to the directions given with the formula for each stain.

Order of Procedure with Vegetable Specimens to be cut in Celloidin.

1. *a.* If the material is fresh, dehydrate in alcohol of gradually increasing strength.
   b. If the material is alcoholic and hard, soak in alcohol and glycerine, equal parts, until soft; if the material is alcoholic and soft, omit this step.
2. Soak 24 hours in thin celloidin.
3. Soak 24 hours in thick celloidin.
4. Imbed in celloidin and immerse in 82 per cent alcohol for 24 to 48 hours.
5. Cut sections, keeping the knife and specimen wet with 50 to 70 per cent alcohol. Bleach the sections, if necessary.
6. *a.* Place in 35 per cent alcohol with the celloidin on the sections.
   b. Stain in Grenacher's Borax-Carmine.
   c. Place in 35 per cent alcohol to which a few drops of hydrochloric acid have been added.
   d. Place in 82 per cent alcohol.

7. *a.* Place in 95 per cent alcohol for not more than 30 seconds.
   b. Place on a glass slip and flood with chloroform, to clear the section. Apply the chloroform at least twice to insure perfect clearing.
   c. Working quickly, before the chloroform can evaporate, drop on pure or chloroform balsam, and cover with a cover-glass.

In place of number 6 may be substituted hæmatoxylin or one of the other carmine solutions, remembering the precautions as to treatment before and after staining. To secure success with the celloidin method, the dehydration must be rapidly accomplished, and chloroform must be used as the clearing agent. The essential oils, such as oil of cloves, oil of cedar, etc., either dissolve the celloidin or cause the section to shrivel, and should not be used by preference, although it is true that they are so used by some workers.



ACME "CONTINENTAL" MICROSCOPE.
Made by Queen & Co., Philadelphia.

FIG. 35.

CHAPTER IX.

SECTIONS OF ANIMAL TISSUES.

Hardening. — Most animal tissues require to be hardened before they can be cut into thin sections. For most tissues ordinary alcohol is the best as well as the most convenient reagent to accomplish this. Care must be taken, however, with delicate tissues, or they will be distorted and contracted because of the shrinkage which results from the fact that the alcohol removes the water from the tissue faster than the alcohol can take its place. With most tissues it is sufficient to begin with 60 per cent and pass through 70 per cent and 95 per cent alcohol. With the more delicate tissues it is best to begin with 40 per cent and pass through 50 per cent, 60 per cent, 70 per cent, and 95 per cent alcohol. The time should be short in the lower grades, as there is danger of maceration if the object is left too long in them.

The specimen to be hardened should be cut into small pieces. Persons not accustomed to the process ordinarily use pieces that are far too large. A piece one-third of an inch square and one-fourth of an inch thick will give more sections than can be used, and it is only a waste of time and of alcohol to use larger pieces. The larger pieces not only do not harden so quickly, but they do not harden so uniformly. Using a piece of one of the softer tissues — a piece of liver, for instance — of the above dimensions, wash it in cold water, and place it in 40 per cent alcohol for one hour; then in 50 per cent alcohol, two

hours; 60 per cent, six hours; 70 per cent, twenty-four hours; 95 per cent, twenty-four hours; and it will ordinarily be sufficiently hardened. The volume of the alcohol should in each case exceed the volume of the specimen many times. Pint bottles with wide mouths are convenient vessels to contain the alcohol. Only one or two pieces of the tissue should be immersed at a time in each of these bottles, and the alcohol should be changed after having been used a few times, the weakest being thrown away and that in each of the other bottles being considered as of the next lower grade, the strongest alone being renewed.

Staining. — Many animal tissues in pieces of the size above mentioned may be stained *in toto* and better results obtained than when the staining is done after the sections are cut. Tissues which are found by experience not to stain well as a whole may be prepared for imbedding and cutting, and be stained after the sections are cut. Carmine and hæmatoxylin stains are most commonly used for animal preparations. The pieces are immersed for twenty-four hours in alcohol of the same strength as the stain to be used. If Kleinenberg's hæmatoxylin is to be used, immerse the pieces in 70 per cent alcohol; if Grenacher's borax-carmine is to be used, immerse in 35 per cent alcohol. It is impossible to give definite directions as to the time required for staining. The pieces must be watched, and when they appear to be deeply stained they are removed to alcohol of the same grade as the stain, made slightly acid with hydrochloric acid. They remain in the acid alcohol twenty-four hours, and are transferred to alcohol of the same strength, and pass through the higher grades to complete dehydration in absolute alcohol.

Imbedding in Paraffin. — The alcohol must now be removed from the specimen to prepare it for the penetration of the paraffin. Place in chloroform for twenty-four hours, adding a little sulphuric ether or oil of cloves to the chloroform; then place for another twenty-four hours in a solution of paraffin in chloroform, at the ordinary temperature; then in some of the same solution on the water-bath, maintaining a temperature of about 130° F. for several hours, or until bubbles cease to rise from the specimen; then in clear paraffin on the water-bath for several hours, at the same temperature. It is then ready for imbedding in the manner described under botanical specimens.

On account of the cost of chloroform, turpentine is most commonly used to remove the alcohol and prepare the specimen for the paraffin, though it is open to the objection that it has a tendency to render objects brittle. The above process need be modified only by substituting the turpentine for the chloroform. The process is, however, sometimes abridged. The object is placed in turpentine, at the ordinary temperature, for some hours, or until it appears clear. It is then placed directly in melted paraffin on the water-bath for several hours, and then imbedded. The temperature of the paraffin should not in any case be allowed to rise much above the melting-point of the paraffin.

Oil of cloves may also be used in connection with turpentine. It drives out the alcohol more thoroughly, and tends to better results. The object is transferred from alcohol to a mixture of alcohol and oil of cloves, half and half, then to clear oil of cloves, then to turpentine, and finally to melted paraffin on the water-bath.

If the casts are spongy, it is an indication of a lack

of thoroughness in the processes. Such casts should be thrown back into the melted paraffin for further treatment. Casts made by the chloroform method will surely be spongy if the chloroform has not been wholly expelled by heating on the water-bath. When the details of this method are carefully attended to, the results cannot be surpassed.

Imbedding in Celloidin. — The method of imbedding animal specimens in celloidin is precisely the same as that detailed under botanical sections, and need not be repeated here. Perfect dehydration before immersing the object in the solution of celloidin is of the greatest importance. The celloidin method gives very perfect results.

Cutting Sections. — The cork bearing the celloidin cast is held in the jaws of the microtome clamp, and the surface of the specimen and the knife are kept wet with 50 to 70 per cent alcohol.

The paraffin cast is held directly in the jaws of the microtome, or is fastened to the roughened surface of a cork by means of melted paraffin. It is an advantage of this method that the sections may be cut dry. A section-smoother should be placed on the knife to prevent the sections curling. If there is a tendency to crumbling, smear the surface of the specimen with collodion before each section is cut and let it dry a minute or two.

Mounting. — To mount a section from a celloidin cast, transfer it to a glass slip, cover it with chloroform, drain, and cover again with chloroform, add pure or chloroform balsam before the chloroform evaporates, and cover with a cover-glass. To mount a section from a paraffin cast, dry a clean slide, place it on the turn-table, and with a camel's-hair brush apply to its centre a very thin coat of collodion fixative, transfer a section from the microtome knife to the centre of the slide with a camel's-hair brush,

and smooth it out by pressing gently on it with a piece of smooth oiled paper. Place the slide in the oven of the water-bath, the temperature being maintained at about 130° F., to evaporate the clove oil from the fixative. The section will then be firmly secured to the centre of the glass slip. Remove the paraffin from the section by pouring upon it a large drop of turpentine. In a few minutes drain off the turpentine and add a fresh drop. It may be necessary to repeat this operation three or four times to remove all traces of the paraffin. Another way is to immerse the slip bearing the section in a bottle of turpentine for fifteen or twenty minutes. Wipe the slip free of turpentine and mount at once in Canada balsam.

If it is desired to mount in glycerine or glycerine-jelly, remove the turpentine by immersing the slip in two changes of 95 per cent alcohol, remove the alcohol by immersing in water, pour a large drop of glycerine on the object, drain it off, and then mount in glycerine or glycerine-jelly.

Order of Procedure with Animal Tissues when the Object is to be stained in toto *and cut in Paraffin.*

1. Harden in alcohol of gradually increasing strength.
2. *a.* Place in 70 per cent alcohol.
   b. Stain in Kleinenberg's hæmatoxylin.
   c. Place in 70 per cent alcohol, slightly acidulated with hydrochloric acid, if overstained; otherwise omit this step.
   d. Wash thoroughly in 70 per cent alcohol, and dehydrate with 95 per cent and absolute alcohol.
3. Soak in chloroform or turpentine for 24 hours.
4. Soak for 24 hours in chloroform or turpentine containing all the paraffin that will dissolve.
5. Place in the same solution on the water-bath, temperature about 130° F., for several hours.

6. Place in clear paraffin on the water-bath at the same temperature for several hours.
7. Imbed in paraffin.
8. Cut sections dry.
9. Fix on glass slip with collodion fixative.
10. Place the glass slip in the oven of the water-bath, temperature about 130° F., until the clove oil is evaporated from the fixative.
11. Remove paraffin with turpentine.
12. Mount in Canada balsam. Or remove the turpentine with alcohol, place in water, then in glycerine, and mount in glycerine or glycerine-jelly.

In place of number 2 may be substituted Grenacher's hæmatoxylin or any of the carmine stains, observing the precautions as to treatment before and after staining.

Order of Procedure with Animal Tissues to be cut in Paraffin and stained after they are cut.

1. Harden in alcohol of gradually increasing strength.
2. Clear by soaking in chloroform or turpentine 24 hours.
3. Place for 24 hours in chloroform or turpentine containing all the paraffin that will dissolve.
4. Place for several hours in melted paraffin on the water-bath, temperature about 130° F.
5. Imbed in paraffin.
6. Cut sections dry.
7. Fix sections on glass slip with collodion fixative.
8. Place slip in the oven of the water-bath, temperature about 130° F., to evaporate the clove oil from the fixative.
9. Immerse the glass slip bearing the section in a bottle of turpentine for 15 or 20 minutes.
10. Remove the turpentine from the section by immersing the slip in two different bottles of 95 per cent alcohol.
11. *a.* Cover the specimen with 70 per cent alcohol.
    b. Immerse the slip in a bottle of Kleinenberg's hæmatoxylin, or put a drop of the stain on the specimen, letting it remain until the section is sufficiently colored.

c. Immerse in 70 per cent alcohol, slightly acidulated with hydrochloric acid, if overstained; otherwise omit.

d. Dehydrate in 70 per cent, 95 per cent, and absolute alcohol.

12. Remove the alcohol with benzole or turpentine (do not use oil of cloves, as it will dissolve the collodion fixative), and mount in Canada balsam. Or, omitting the dehydration in number 11, place in water, then in glycerine, and mount in glycerine or glycerine-jelly.

Order of Procedure with Animal Tissues to be stained in toto *and then imbedded in Celloidin.*

1. Harden in alcohol of gradually increasing strength.
2. Stain in hæmatoxylin or carmine with the proper treatment before and after staining.
3. Dehydrate in 95 per cent and absolute alcohol.
4. Soak in thin celloidin for 24 hours.
5. Soak in thick celloidin for 24 hours.
6. Imbed in thick celloidin on a cork in the manner already described.
7. Immerse in 82 per cent alcohol for 24 to 48 hours.
8. Cut sections, keeping the knife and specimen wet with 50 to 70 per cent alcohol.
9. Place a section on the centre of a glass slip and cover it with 95 per cent alcohol. Immediately drain off the alcohol and cover with chloroform. Repeat the chloroform twice, and before the chloroform can evaporate mount in chloroform balsam or pure balsam.

Order of Procedure with Animal Tissues to be imbedded in Celloidin, the Sections to be stained after cutting and fixed in Position on the Glass Slip.

1. Harden in alcohol of gradually increasing strength to perfect dehydration in absolute alcohol.
2. Soak in thin celloidin for 24 hours.
3. Soak in thick celloidin for 24 hours.
4. Imbed in celloidin.
5. Immerse in 82 per cent alcohol for 24 to 48 hours.

6. Cut sections, keeping the knife and specimen wet with 50 to 70 per cent alcohol.
7. Stain in hæmatoxylin or carmine.
8. Place in 95 per cent alcohol not more than 30 seconds.
9. Place on the centre of a glass slip, and fix in position by placing on the section just enough of a thin solution of bleached shellac in alcohol to cover it.
10. Place in the oven of a water-bath, temperature about 130° F., to evaporate the alcohol.
11. Clear in chloroform and mount in Canada balsam.

CHAPTER X.

CHEMICAL CRYSTALS.

THE preparation of chemical crystals, either for temporary examination or for permanent mounting, is both easy and attractive. A very large number of chemical substances crystallize in most beautiful forms from solutions. The same chemical often gives crystals of very different forms when treated in different ways. For many substances it is necessary only to make a solution in water, place a drop of the solution on a cover-glass or glass slip, and allow it to evaporate at the ordinary temperature of the room. The solution may be made in a small test-tube or in a watch glass. Very small quantities only of the chemicals and of the solvent should be used, as but a small drop of the solution is needed. Another method of making the solution is to place a drop of water or other solvent on a cover-glass or glass slip, add a few particles of the chemical, and apply gentle heat.

Different forms of crystals are obtained from solutions of different strengths. If saturated solutions do not give good crystals, especially if the results are massive and coarse, try weaker solutions. Some of the most delicately beautiful crystals form from very weak solutions. Other solvents than water may be used, as alcohol, ether, chloroform; and the greatest variety of interesting forms of crystals results. The forms also vary according as the evaporation is rapid or slow. The best results are obtained from some substances by retarding the evaporation, by

placing the cover-glass with the drop of solution on it in a damp, cool cellar, until the crystals are formed; sugar is an example of such substances. Crystallization may also be retarded by adding to the solution gelatine in varying quantities. A few strips of gelatine are placed in a test-tube with a considerable volume of water and, after soaking some minutes, are dissolved by the application of heat. A little of this solution is poured into another test-tube or into a watch glass, and the chemical under treatment is dissolved in it. The crystallization is allowed to take place at the ordinary temperature of the room. Many of the common chemical salts give excellent results when treated by this method.

Some solutions need to be evaporated by the application of artificial heat or by exposure to the direct rays of the sun. Potassium chlorate, potassium chromate, potassium dichromate, potassium oxalate, potassium permanganate, potassium iodide, sodium chloride, cobalt chloride, ammonium nitrate, copper nitrate, oxalic acid, cadmium sulphate, iron sulphate, barium chloride, nickel nitrate, and many other common chemical salts may be dissolved in water and treated by this method.

In other cases no solution is made, but a small amount of the solid substance is placed on a cover-glass and held a few inches above a flame until it melts. The cover-glass is then withdrawn from over the flame and, upon cooling, the substance recrystallizes in its own water of crystallization. Salicine, santonine, and benzoic acid are substances which give especially fine results by this method.

For temporary examination a solution of the chemical salt is made by one of the methods described, and a few drops placed on a glass slip previously somewhat warmed. The slip is placed on the microscope stage, slightly inclined,

and the crystallization watched with a low power, — two inch, one inch, or three-fourths inch objective, — under polarized light. If crystallization does not begin within a few minutes, the slip is again slightly warmed. A cover-glass should not be placed over the crystals in this examination.

Interesting and instructive crystals are also formed by filling shallow cells, made by cementing rings to glass slips, as described in Chapter VI., with a saturated solution of the chemical under examination and setting aside, protected from the dust, until the crystals are formed. A thread laid diametrically across the cell, just touching the liquid, will often cause the crystallization to start along this diameter and so be in a convenient part of the cell for observation. The crystals formed in a considerable quantity of liquid are much more characteristic of the substance than the tabular crystals, which result when a thin layer of liquid crystallizes on a flat surface. For this reason no study of the crystallization of a substance is complete until an examination has been made of the crystals formed in the "mother liquor." The crystals may be permanently preserved in these cells. After the crystals have been formed, fill the cell even full of the saturated solution of the substance used, place the slip on the turn-table, and run a fresh ring of shellac cement on the outer edge of the cell, breathe on a cover-glass, place it over the cell, press it gently down, and see that there are no air bubbles. When dry, finish as already described for cell mounts. Slides thus prepared should be handled carefully, to prevent displacement of the crystals by sudden jars.

For permanent mounting it is better, as a rule, to form the crystals on the cover-glass rather than on the slip. A cover-glass is cleaned, slightly warmed, a drop of the solu-

tion of the chemical in water, gelatine-water, alcohol, ether, chloroform, or other solvent, is placed on the centre, and the crystals are allowed to form. It is not to be expected that every trial will give results worthy of preservation, but a little patience will meet with sure reward. The crystals may be mounted in various ways. Carpenter recommends castor oil as the best preservative. Balsam mounting is much easier, and is most excellent for those substances that will stand the balsam without dissolving. The following substances, crystallized by this method, are permanent in pure balsam: potassium chlorate, potassium ferrocyanide, potassium ferricyanide, potassium oxalate, potassium chromate, potassium dichromate, copper sulphate, iron sulphate, tartaric acid, ammonium oxalate, borax, salicine, cane sugar, and doubtless many others.

In mounting, a drop of pure Canada balsam is placed on the centre of a glass slip, the cover-glass on which the crystals have formed is inverted over it, centred, and gently pressed down, care being taken to disturb the crystals as little as possible. The balsam must be allowed to harden at the ordinary temperature.

A method applicable with a few substances, and giving most beautiful results, is to place a small drop of balsam on a slide, heat until the volatile matters are driven off, then add a little of the solid chemical, and heat again until solution of the chemical in the balsam is effected, put on a warm cover-glass properly centred, and set it aside to cool. This method possesses the advantage of effecting the mounting with a minimum of trouble, and the added advantage that the slide may, immediately upon cooling, be freed from the superfluous balsam and receive the finishing rings of cement. Sulphonal and salicine give excellent results by this method.

Most chemical crystals may be preserved in dry mounts. A shallow cell is necessary, best formed of shellac cement, though paraffin cells are popular for this purpose. It is best to have on hand a quantity of cells ready prepared, of various depths, and to use in each case one that is but little deeper than the thickness of the crystals to be mounted. Crystals of deliquescent and efflorescent substances, if mounted dry, must be mounted and thoroughly sealed immediately after they are formed and before the air has had time to act upon them.

In the examination of crystals polarized light should be employed. Even crystals belonging to the cubical system are brought out more clearly in some positions of the Nicols than they are in ordinary light. These crystals do not polarize, of course, unless, as sometimes happens, the film is subjected to a strain while the crystals are forming. Crystals of other systems polarize with endless variety of colors. The selenite plate accompanying the polarizing apparatus, or in lack of this a selected film of mica, placed on the stage under the slide, gives a colored field which aids in bringing out the forms and colors for crystals which do not happen to be of the right thickness to polarize well of themselves; but the use of the selenite detracts rather than adds to the interest of other crystals which are of suitable form and thickness to give brilliantly beautiful colors without adventitious aid. In the course of the examination, the rotating Nicol should, of course, be turned to obtain different color effects, and to enable the observer to watch the change of each color to its complementary.

It is hardly necessary to give an extended list of chemical substances from which crystals may be formed. Many nitrates, some sulphates, most chlorates, oxalates, citrates, and tartrates form polarizing crystals.

PETROGRAPHICAL MICROSCOPE.
Made by Bausch and Lomb Optical Co., Rochester, N.Y.

FIG. 36.

CHAPTER XI.

ROCK SECTIONS BY SIMPLE MEANS.

WITHIN recent years the study of the microscopic structure of rocks has come to be recognized as an important feature of geological investigation. The microscope helps in the determination of the soundness and of the purity or impurity of minerals, and aids in deciding upon the nature and character of the impurities. Before the application of the microscope to rock sections, chemical analysis was the only means of determining the composition of rocks. In many instances where chemical analysis fails, the microscope decides with certainty on the mineral composition; and in the case of certain minerals, nearly all that we know of their origin has been learned from the microscopical study of their sections. It is true that the microscope cannot settle all questions, but it has already largely superseded chemical analysis where the end in view is simply the determination of the mineral ingredients; and in most cases where a quantitative determination of the minerals is to be made by chemical processes, the microscope lends important aid in making the preliminary qualitative determination. The value of the microscopical study of rocks is being more and more recognized.

The practical use of the microscope for these purposes involves a vast amount of study — study of great interest, and which, on account of the newness of this line of research, offers peculiar attractions. It is not the presumption of this chapter to give an exhaustive treatment of

petrology, but to state simple methods for the preparation of thin sections of rocks for microscopical study, with a few hints at the methods of studying these sections.

Specialists have devised special apparatus for the preparation and examination of rock sections, which is quite out of the reach of most private workers on account of the expense involved. A great deal may be done with the usual microscopical outfit. Many sections of rocks yield very interesting results when examined by ordinary light; for the examination of most rocks polarized light is, however, essential. A revolving stage gives an additional means for their study. A plate of selenite or mica gives a colored field which, in many cases, is a help in bringing out the characteristic features of minerals. This outfit of apparatus is all that is really necessary for a large amount of work in this interesting line of microscopical investigation.

If one has the use of a lathe, slices of rocks may be sawed by means of a thin disk of iron or copper charged at its edge with diamond dust, and these slices may then be ground down on emery wheels. This method of working, however, involves too much expense for most private workers.

Very simple means suffice for much interesting and instructive work in grinding rock sections. All that is needed is a smooth piece of iron — an old stove cover or a piece of smooth sheet iron will do — some coarse emery, say, No. 120, or even coarser for many rocks, some flour of emery, bits of window glass, and some Canada balsam.

For a specimen to begin with, granite is good. Select a granite that is firm and sound. With a hammer chip off a piece about half an inch square and as thin as possible. Place a little of the coarse emery on the iron plate,

together with a little water. Holding the specimen firmly with the fingers, grind one side flat. Wash off the coarse emery, and continue the grinding with flour of emery in water on a plate of window glass until the surface is quite smooth. Wash the specimen again and dry it. Now place a few drops of Canada balsam on a piece of window glass about an inch square, and heat to drive off the volatile portions of the balsam. A spring clothes-pin makes an excellent holder for this operation. The balsam, after cooling, should be quite hard and firm, but not brittle. Care should be taken not to let the balsam take fire. Place the specimen, smooth side down, in the warm balsam, and press down upon it gently until the balsam has cooled, seeing that no air bubbles are enclosed between the specimen and the piece of glass. When the balsam is cold, the specimen will be firmly cemented to the glass. Using the glass as a holder, continue to grind until the specimen is reduced in thickness to such a degree that light is readily transmitted through it.

The specimen should be examined at intervals under the microscope to watch the progress of the work. Wash it free of the emery, place a drop of water on it, and cover with a cover-glass. As the specimen becomes thin, care should be taken that the balsam is not worn away up to its edge. If this happens, the specimen may be rapidly broken away. The remedy is to clean and dry the specimen, place a little fresh balsam on it, drive off the volatile matter, and let the balsam set around the specimen again. The latter portion of the grinding should be done in the flour of emery on a plate of glass, as before.

When the specimen has been reduced to the desired degree of thinness, place on it a large drop of Canada balsam, and heat until the object is released from the piece

of glass. Then with a needle carefully slide the specimen into a watch glass of alcohol, and let it remain until the balsam and contained dirt have been removed from it, draining off the alcohol and adding a fresh portion once or twice, if necessary. The specimen may now be lifted by means of a camel's-hair brush, and placed on the centre of a microscope slide, dried carefully in gentle heat, and mounted in Canada balsam.

Very brittle specimens are liable to be broken in the process of removal from the glass holder to the alcohol. This may in a measure be guarded against by placing a cover-glass over the specimen after the fresh balsam has been added. Heat and remove specimen and cover-glass together to the alcohol. The cover-glass will also serve to support the specimen in removing it from the alcohol to the slide. See that the section is on the centre of the cover-glass, remove, drain, and dry. Have ready a slide with a drop of balsam on its centre, invert the cover-glass and specimen over the balsam, and centre. In the case of excessively brittle specimens, it is best to omit the washing in alcohol. Remove the specimen, supported by a cover-glass, directly to a glass slip on which a drop of balsam has been previously placed.

The amount of material available for the microscopical study of rocks is unlimited. The dark rocks are, in general, so opaque that they must be ground to extreme thinness unless the sections are to be examined by reflected light; but any of the lighter colored stones yield good results. A cabinet of minerals affords numerous specimens for instructive study. Fossils often yield most beautiful specimens. Silicified wood, for instance, though very hard and requiring much patience in grinding, amply compensates for the labor by revealing the minute plant struct-

ure as plainly as a piece of a growing tree. Pieces of silicified wood are sometimes found which, by a blow from a hammer, cleave into thin films, ready to mount for the microscope. The study of the microscopic structure of fossil plants, though until recently but little attention was paid to it, is now recognized as a valuable adjunct to the study of growing plants. Indeed, it is only by the study of fossil specimens that the historical development of modern plants can be investigated.

All rocks of crystalline structure afford much interesting material. When a section of such a rock is viewed by means of polarized light, the variegated coloring is very beautiful. As the analyzer is turned, each color changes to its complementary. The labor bestowed in grinding rock sections is amply repaid if no further object is sought than to obtain beautiful and interesting objects for a cabinet.

In the study of the sections of a crystal in a rock section, it is to be remembered that the form of the section will depend upon the direction in which the section was cut. A cubical crystal might, for instance, give any of the following shapes: an equilateral, an isosceles, or a scalene triangle, a square, an irregular quadrilateral, or a parallelogram. The same rock section will often give several different sections of crystals of the same mineral. At the most, the study of a small number of sections of the same rock will usually give quite a definite idea of the shapes of the crystals that it contains. In some cases, however, the crystals are much changed in shape by compression in the rock mass, so that the study of the crystalline structure is made complicated.

If the revolving stage of the microscope has a graduated edge, and the tube of the instrument has centring

screws, so that its axis may be made to pass accurately through the centre of the stage, the angles of the crystals may be measured. An eye-piece with crossed hairs is used. The point of intersection of the hairs is made to coincide with the vertex of the angle to be measured, and one of the hairs is made to coincide with one side of the angle. The stage is then revolved until the same hair coincides with the other side of the angle, and the number of degrees through which it has been turned is read from the graduated edge of the stage. The measurement of the angles affords valuable means for the identification of crystals.

The presence or absence of cleavage is another means of identifying minerals. The cleavage lines come out very plainly under the microscope, even when they cannot be made out in the massive structure with the unaided eye. The angle formed between the cleavage planes determines at once several different minerals. It is to be remembered that the direction in which the section is cut relatively to the face of the crystal modifies the size of the angle, so that it is important to determine in what direction the section is made with reference to the face of the crystal before relying upon the angle to decide what the mineral is.

In studying sections of crystals under the microscope, the lack of purity is very noticeable. Minerals which to the unaided eye seem to be perfectly pure are often found to contain large amounts of other substances, either crystalline or amorphous. Crystals enclosed within crystals are very common; these crystals are sometimes large enough to be determined; sometimes so small that identification is impossible. Cavities are also frequently found which contain a small bubble of water, or of liquid car-

bonic acid, or a small cube of common salt, which has crystallized from the supersaturated salt water that was enclosed in the rock at the time of its formation.

As we have seen in Chapter III., polarized light furnishes the means for determining whether a substance is singly refractive or doubly refractive. If some portions of a rock section, viewed in polarized light, do not restore the light when the Nicols are crossed, and do not give color effects when the analyzer is turned, or when the stage is rotated, it is at once known that they are singly refractive or isotropic. If, under these conditions, the light is restored or color effects are produced, these portions of the section are doubly refractive or anisotropic.

The singly refractive portions of the section are either amorphous, or are crystals of the isometric system, or are sections of crystals of other systems cut perpendicularly to an optic axis. Amorphous substances are readily distinguished from crystalline substances, from the fact that the latter almost invariably show either a definite polygonal outline or cleavage lines. Isometric crystals may be distinguished from crystals of other systems cut perpendicularly to an optic axis by means of convergent polarized light. To obtain the convergent polarized light, petrographical microscopes are provided with a convex lens, to be mounted just above the polarizer, or with some other means of converging the light. If a section of an isometric crystal, cut in any direction, is viewed in this light, nothing more is noticed than when ordinary light or parallel polarized light is used. Sections of crystals belonging to the tetragonal and hexagonal systems cut perpendicularly to the vertical axis, which, as we have seen, act like isometric crystals in parallel polarized light, when viewed in convergent polarized light, show rings of prismatic colors and

a distinct black cross when the Nicols are crossed. This cross remains stationary when the crystal is revolved. It is changed to a white cross as the analyzer is revolved, while at the same time the colors of the rings change to their complementaries. These basal sections of hexagonal and tetragonal crystals are further distinguished by the outline of the section, which is a polygon of some multiple of three sides for the hexagonal crystals, and of four or eight sides for tetragonal crystals. There is but one direction in hexagonal and tetragonal crystals, viz. that parallel with the vertical axis, along which parallel polarized light can pass without modification, and it is only when convergent polarized light passes in this direction that the rings of prismatic colors and the black cross result. Parallel polarized light passing in any other direction through crystals of these systems is again polarized. The field is dark when the vertical axis of the crystal corresponds to the planes of vibration of either Nicol, light in other positions of the crystal, the Nicols being crossed. Color effects are produced as the crystal is rotated or the analyzer turned. These crystals are uniaxial.

In the case of crystals of the orthorhombic, monoclinic, and triclinic systems, there are two directions in which parallel polarized light passes unmodified. In all other directions it is doubly refracted. These crystals are biaxial. A section perpendicular to either of the optic axes behaves like an isotropic substance in parallel polarized light. In convergent polarized light, more or less elliptical rings of prismatic colors are seen; but instead of a black cross, as in basal sections of crystals of the tetragonal and hexagonal systems, when the Nicols are crossed, a single bar of black will be seen. A further distinguishing characteristic is the fact that when the

crystal is revolved the black bar revolves in the opposite direction.

If a section of a biaxial crystal, cut perpendicularly to the line which bisects the acute angle formed by the optic axes, is viewed in convergent polarized light with the Nicols crossed, a series of elliptical rings of prismatic colors with two black hyperbolas will be observed in one position of the crystal. If the crystal is rotated, the hyperbolas give place to a black cross with unsymmetrical arms. If the analyzer is turned, the black hyperbolas change to white, and the prismatic colors to their complementaries.

Any section of an orthorhombic crystal, placed so that any one of the three crystallographic axes is parallel with the planes of vibration of the light, is dark between crossed Nicols. Some sections of monoclinic crystals are dark between crossed Nicols when a crystallographic axis is parallel with the planes of vibration of the light, while others restore the light. In the latter case, if the section is revolved through a certain number of degrees, differing in different crystals, the field becomes dark. Sections of triclinic crystals are never dark between crossed Nicols when a crystallographic axis is parallel with the planes of vibration of the light. On turning the crystal through some degrees, the field becomes dark. The number of degrees through which the crystal must be turned in these two cases is a valuable aid towards determining the crystal. The position of the crystallographic axis can usually be determined by the outlines of the crystal or by cleavage lines.

The determination of the system to which a crystal belongs is, of course, an important step towards its identification. In some cases this determination is quite easy;

in other cases it is hardly necessary to say that it is most difficult. Systematic study of the crystals in rocks presupposes extensive training in crystallography, calls for the keenest powers of observation, and the highest exercise of the reasoning faculties. What has been here said is intended merely to suggest to the interested beginner some of the methods employed in the microscopical examination of rock sections.

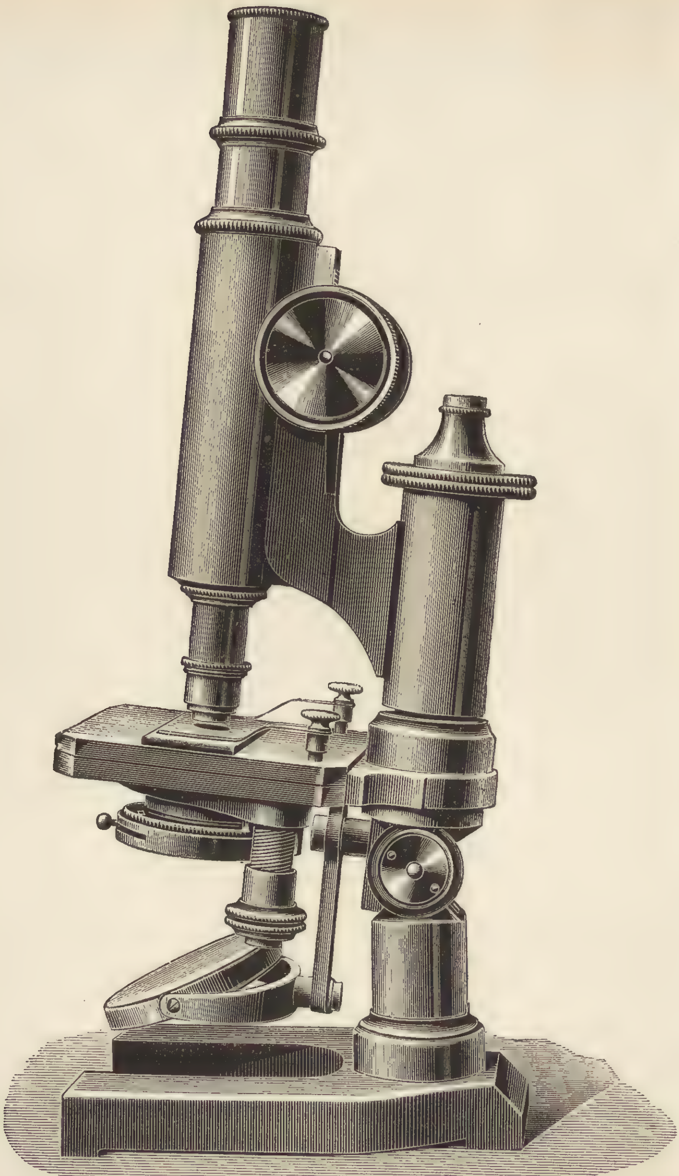
Cleavage lines, angles of crystals, outlines of crystals, determination of the system to which crystals belong, enclosure of crystals and amorphous substances in crystals, cavities containing air, water, or liquid carbonic acid, effect on the light, color of the crystals, association of minerals, are, then, some of the characteristics relied upon in the identification of the components of a rock.

Rolling Sand. — Although not strictly belonging under the heading of this chapter, a slide of rolling sand is properly classed with rock specimens. Such a slide is not very difficult of preparation, and is a most interesting addition to one's collection. Clean quartz sand from the seashore or the beach of a pond or stream is required. A cell of such depth that the sand is free to move after the cover-glass is in position is used. The mounting fluid is glycerine slightly diluted with water.

Place a small quantity of the sand in a cell, fill the cell full of the fluid, and see that there are no air bubbles clinging to the sand or to the edges of the cell. Run a ring of shellac cement around the outside of the top of the cell, and put the cover-glass in position. See that no air bubbles are enclosed. Clean and finish the slide in the manner already described for glycerine mounts.

For examination, slightly incline the stage of the microscope, having the Nicols crossed. Hold the slide edge-

wise for a moment until the sand runs down to one side. Place the slide in position on the stage with the sand uppermost. As the sand now rolls slowly down (it appears, of course, to roll up hill), a most magnificent display of color changes is seen as the different positions of the grains of sand bring different thicknesses of the quartz between the Nicols. The specimen is very showy.



LEITZ'S STAND II.
Franklin Educational Co., Agents, Boston.
FIG. 37.

CHAPTER XII.

THE STUDY OF BACTERIA.

In the study of bacteria the microscope plays only a subordinate part, yet without it this study would be impossible. The numerous methods of preparation, culture, and isolation of bacteria lead up to an examination under the microscope. Some account of such methods in their simpler forms would seem then to have a place in these pages. Much may be done with powers of the microscope not higher than four or five hundred diameters. The processes employed may be learned, and very definite ideas of what bacteria are, and the means used by specialists in their investigations, may thus be acquired. For the more satisfactory study of such preparations, however, a substage condenser and the very highest powers of the microscope are necessary, used with the highest degree of manipulative skill. It is not to be expected that the finer points of detail will be seen except by these means.

The beginner is cautioned to remember that bacteria are the minutest organisms in nature, and that they can be seen under moderate powers of magnification only by the closest observation. In looking for them, focus sharply with the fine adjustment and expect to see exceedingly minute rods, spheres, and curves, or, in the case of filamentous forms, very fine segmented threads. These are all highly transparent, unless they have been submitted to the action of some staining fluid. They may easily be

overlooked, but a little persevering observation will cultivate the eye to detect them.

The name *bacteria* is applied to countless numbers of forms of vegetable organisms. They exist everywhere. They are the active cause of putrefaction and of fermentation. The decay of all animal and vegetable substances is occasioned directly by the rapid multiplication of different kinds of these organisms. Many chemical changes in organic substances are due to their agency. The souring of milk, for instance, is caused by the action of bacteria, which convert the sugar of the milk into lactic acid, and this into butyric acid and other products. The development of flavors in the ripening of cheese is brought about through the action of bacteria. It is believed that bacteria play an important part in the stomach and intestines in the digestion of food.

Under favorable conditions the multiplication of bacteria is enormously rapid. As long as they can obtain an abundance of food material of the right nature, they multiply by fission; but if food material fails, or the conditions for this kind of development are otherwise not right, a clear round or oval spot appears in each individual organism of many of the species. This is the resting-spore. It continues to live without nourishment after the rest of the organism has disappeared; it resists drought and cold. These spores, in the dry condition, are taken up by the air, and are afterwards deposited on all exposed surfaces. When they are brought in contact with suitable nourishing material they spring into life with great vigor.

The reproduction is effected by the splitting of the cells. Hence the name, Schizomycetes, or fission-fungi. There are, however, good reasons for believing that they do not belong with the fungi. Various forms occur. All

are enclosed in a structureless gelatinous membrane. One general division includes minute spherical cells which divide by a wall running across in one direction, or by walls crossing one another; these receive the general name of Micrococcus. Another division, including straight, rod-like forms, receives the name Bacillus. If the rods are very short and thick, with somewhat rounded ends, the name Bacterium is employed. Slender, long rods are called Leptothrix; thick, long rods, attached at one end of the filament to some supporting object, are called Beggiatoa. Crenothrix is a name used for a form of large rods having about them an especially distinct gelatinous envelope. Branching forms are called Cladothrix. The cells of the rod-like forms multiply by first increasing in length, and then throwing a partition transversely through the middle of the cell, the new cells formed repeating the process indefinitely, giving rise to chains of cells connected by a highly transparent membrane. The individual cells are so small that the minute structure is very hard to study. So far as is known, each cell is filled with protoplasm of uniform composition, but differing somewhat in different species. The belief that protoplasm is present is based upon the fact that only the more opaque portions are stained when iodine is run under the cover-glass. Of the coiled forms, those that are short, stout, and have but few coils are called Spirillum; those that are long, slender, and have many coils are called Spirochæta. Vibrio is a name applied to loosely curved and segmented forms. In each of these general divisions are innumerable individual forms, many of which have been studied and given particular names. It is to be remarked, however, that there is more or less of confusion in the use of these names in the literature of bacteriology.

The prevailing colors of bacteria are gray, yellowish white, and white. Some species are bright, and are readily recognized in masses as red, blue, green, or brown. In some cases the coloring matter exists in the protoplasm; more frequently it is found in the envelope of cellulose-like material which surrounds the protoplasm. The coloration arises from the power which bacteria possess of chemically separating the substances in which they thrive, and of forming new compounds, or upon their power of absorbing ingredients from the surrounding substances. Crenothrix and Cladothrix are of a distinct brown color, due to the oxide of iron thus absorbed from the water in which they thrive. The yellow color of Beggiatoa is due to sulphur, which has in a similar way been separated from the sulphur water in which these bacteria grow. The blood-like drops which form on bread and some other substances when left in a moist atmosphere under suitable conditions of temperature are simply masses of bacteria, the *Bacillus prodigiosus*, which have developed from germs deposited from the atmosphere. The blue color which sometimes under favorable conditions develops in milk is explained in a similar way. The green color which develops on cheese is another example. It is characteristic of bacteria as an order of plants that they are devoid of chlorophyll. Yet there are exceptions to the rule, — at least some forms give the characteristic blue color when treated with iodine solution, showing that starch, the product of chlorophyll, is present. The color is in some cases an important aid towards the identification of the bacteria.

In connection with color may be mentioned the phosphorescence seen at times on the ocean and on decaying wood. It is caused, in some cases, by different kinds of

bacteria, which have the power of separating phosphorus from some of its compounds.

Some forms of bacteria are endowed with the power of motion, or at least have this power at certain stages of their existence; other forms are incapable of moving. It is probable that in the case of some of the moving forms the motion is effected by the contractions of the protoplasm of the cell. In other forms there are hair-like appendages, either from the ends or sides of the cell. These cilia, or flagella, are believed to consist in some cases of protoplasm, and to be organs of motion; in other cases it is believed that the cilia are merely prolongations of the membrane enveloping the cell, and that they have nothing to do with the production of motion. These appendages are naturally difficult to make out, but the number of forms possessing them is now known to be very large.

The observer is cautioned to remember that almost all finely divided solid matters suspended in a fluid exhibit a peculiar oscillatory motion called the Brownian movement. The study of the Brownian movement made in Chapter IV. should be recalled, as many kinds of bacteria exhibit this movement. Careful observation will readily enable one to distinguish between the progressive movement from place to place of the really motile bacteria and this purely mechanical vibratory movement. The hay bacillus, described later, affords good material for the study of both these movements. Another movement about which one might be mistaken is the motion of Vibriones. A filament of Vibriones is shaped like a corkscrew, and the movement is believed to be a revolution on the axis of the filament. Under the microscope it appears by an optical illusion to be a snake-like, undulatory motion. In the

case of Spirochæta and Spirillum, the serpentine motions are believed to be real.

Many forms of bacteria are perfectly harmless. In fact, they are essential to life either as a direct agent in digestion, as already noted, or by their action in the decomposition of animal and vegetable substances, and the preparation of the soil for the production of plant life. With the air, the water, and with our food, we receive countless numbers of bacteria into our systems, where they are either inert or perform the functions for which they were designed in the economy of nature for our well-being. The investigations of recent years have equally well established the fact that other forms are pathogenic. It now scarcely admits of question that cholera, typhoid fever, diphtheria, consumption, and many other diseases, are caused and propagated by particular bacteria.

With these introductory remarks, which are intended to call the attention of the beginner to the nature and importance of the subject, we will turn our attention to the methods employed in the study of these exceedingly minute organisms. As already intimated, culture methods must be employed in the satisfactory study of most forms of bacteria. The remainder of this chapter will have to do with forms which can be studied without special cultivation, and with those which can be cultivated by very simple means. The following chapter will describe the methods now employed in the cultivation, isolation, and examination of forms which cannot be so directly studied.

Bacteria of the Fur of the Teeth. — Scrape a little of the fur from the teeth, and place it on a cover-glass in a thin, even layer with a small drop of water. Invert the cover-glass on a slide, and examine with the highest power at command. Several forms of bacteria will undoubtedly be

found. Minute spheres of Micrococci are clustered together; a spiral form, *Spirilla dentium*, or, as it is sometimes called, *Spirochœta denticula*, darting back and forth with characteristic movements, may be present; possibly the comma bacillus of the teeth, a form curved like a comma (,) and closely resembling the comma bacillus of Asiatic cholera, will be seen in active motion; larger than any of these, straight, rod-like forms will be found, a variety of Leptothrix. If a little iodine solution be run under the cover-glass, the protoplasm in the largest rods will contract sufficiently to show that the rods are segmented. Possibly a blue color, due to the presence of starch granules, will be seen. With the exception of comma bacillus, the bacteria of the mouth cannot be cultivated by the ordinary means.

Putrefaction Bacteria. — Expose to the air of a living-room at the ordinary temperature pieces of moist bread, boiled egg, boiled potato or turnip, for twenty-four to forty-eight hours. Small spots, either translucent or colored, will form. These are colonies of bacteria that have developed from germs deposited from the air upon the substance used.

Transfer a little from one of these spots to a cover-glass with a small drop of water, and examine as already described. Numerous rod-like and spherical bacteria will be seen, many of the rods in active motion.

For further study, boil some green leaves in a flask. Almost any kind of leaves will do. Cabbage or lettuce leaves are good. In another flask boil some ground peas. Filter each solution after an hour, and set aside open at the ordinary temperature. In about forty-eight hours skin-like formations will be seen on the surfaces of the liquids in the flasks. This formation is called a pellicle.

A little of this is examined under the microscope in the manner already described.

Again, soak some hay in water at the ordinary temperature for four or five hours. Filter the infusion into a flask, and boil gently for a few minutes. Plug the mouth of the flask with a wad of cotton-wool, and place it aside at the ordinary temperature for twenty-four or forty-eight hours. A pellicle forms. This is formed by the hay bacillus, *Bacillus subtilis.* The germs of this bacillus are able to withstand boiling for a few minutes. Other bacteria present have probably been killed by this treatment. We have then a pure culture, as it is called, of this variety. The cotton-wool plug is an efficient filter for the air that enters the flask, no germs being able to pass through it. If the boiling was not continued long enough, other forms of bacteria will also be present.

If a bit of the pellicle be examined at the right stage of development, filamentous forms will be seen imbedded in a transparent, gelatine-like substance. In the active, swarming state, the *Bacillus subtilis* divides into filaments of twos, fours, and eights, with flagella at the ends of the filaments. These bacteria will not stain by the ordinary methods.

For still another study, place some Spirogyra, a water plant described in Chapter V., in a flask of water, and allow it to stand until decomposition begins. From time to time examine a drop of the water. Very lively motile coiled bacteria, *Spirochæta plicatalis*, are likely to be found. These bacteria are common in any stagnant water containing decaying organic matter. In the same water the straight, comparatively thick rods of *Beggiatoa alba* may be found. These are filamentous. The protoplasm is granular in appearance, due to the particles of sulphur.

That these granules are sulphur may be proved by allowing the preparation to dry and then running a little carbon bisulphide under the cover-glass. This will dissolve the sulphur, and the granulation will become less distinct.

In all of these cases, the masses taken from the pellicle show the bacteria in the zoöglœa stage. The pellicle is composed of motionless bacteria surrounded by a gelatine-like, transparent substance which is a product of their growth. If we take our specimen at the right time, we have the bacteria in the active, swarming stage, when they are in active motion. At the proper stage of development, we may see the resting-spores. In order, then, to form correct ideas of bacteria, one should make frequent examination, to see them in the different stages of their development.

Cover-Glass Preparations. — In the study of the above, as well as of all other bacteria, it is of advantage to make a so-called cover-glass preparation. A very thin layer of the medium containing the bacteria is placed evenly on a cover-glass. This is best effected by placing a minute drop on a cover-glass and laying another cover-glass on this. The two cover-glasses are now gently rubbed together, and then separated by sliding them apart. They are then dried at the temperature of the room, or by holding them in the forceps some distance above a gas flame, being careful not to scorch the film. The bacteria are now stained by placing on each cover-glass a solution of fuchsin, Bismarck brown, methyl violet, or other staining solution. This is allowed to act from ten to twenty minutes, or longer. The dye is now washed off with distilled water; and, if there has been considerable diffuse staining of the ground tissue, the cover-glass is washed in alcohol. The cover-glass is now dried as before, and a small drop

of oil of cedar, turpentine, or xylol is placed upon it. It is then inverted on a glass slip, and is ready for examination.

In some cases double staining is of advantage. After the surplus of the first stain has been washed away, as above, the cover-glass is not dried, but is treated with a contrast stain from ten to twenty minutes, washed, treated with alcohol if necessary, dried, and mounted in oil of cedar, turpentine, or xylol for examination. Fuchsin and Bismarck brown, Bismarck brown and methyl violet or methyl blue, give good results. These stains may be used in water solution or in alcoholic solution, with the exception of Bismarck brown, the alcoholic solution of which deteriorates on standing. A nearly saturated solution in alcohol of the other dyes may be kept on hand and diluted with about twice their bulk of water, and filtered, at the time of using. As a rule, the bacteria take only one of the colors. The object of the second color is to stain the ground tissue, and so set forth the bacteria by contrast.

A cover-glass preparation made as above may be permanently preserved by simply draining off the oil of cedar, turpentine, or xylol, and mounting in xylol or benzole balsam. Do not use chloroform balsam, as the chloroform dissolves the aniline dye. The bacteria are very much more distinctly seen when mounted in balsam than when mounted temporarily in oil of cedar, etc.

The Bacillus Tuberculosis. — The *Bacillus tuberculosis* is a slightly curved non-motile rod, containing frequently from two to six spores. These spores are very difficult to make out. The rods are from 1.5 μ to 3 μ long and from .2 μ to .3 μ thick. Numerous methods have been devised for the demonstration of the *Tubercle bacilli*. Two reliable methods are here given.

Ehrlich's Method.

Mix 1 c.c. of aniline oil in 20 c.c. of distilled water, and filter. To this add an equal volume of a saturated solution of fuchsin in alcohol. A cover-glass preparation of sputum is made with this as a first stain. The stain is allowed to act for half an hour or longer. The cover-glass is washed first in 33 per cent nitric acid, and then in distilled water. Nitric acid is used because it discharges the color from all bacilli likely to be present except the *Tubercle bacilli.* The bacilli are stained red. As a contrast stain, either methyl blue or Bismarck brown is now applied in saturated alcoholic solution. The ground tissue will be stained blue or brown, the bacilli remaining red. Wash in distilled water and in alcohol, clear in xylol, and mount in xylol balsam.

The Ziehl-Neelsen Carbol-Fuchsin Method.

A cover-glass preparation of sputum is treated with a staining solution prepared by dissolving one part of fuchsin in ten parts of absolute alcohol and then adding 100 parts of a 5 per cent solution of carbolic acid in water. This solution may be heated in a watch glass until it steams, and the prepared cover-glass floated on it, prepared side down, from three to five minutes; or the solution may be used cold, in which case it should be allowed to act for twenty-four hours. The cover-glass is then washed in alcohol and in 25 per cent sulphuric, nitric, or hydrochloric acid. The acid removes the color from everything except the bacilli. Wash again in alcohol, and then in a saturated water solution of lithium carbonate to fix and intensify the stain. As a contrast stain, use

a saturated solution of methyl blue in water, allowing it to act ten or fifteen minutes. Wash and dry the cover-glass, clear in aniline oil or xylol, and mount in xylol balsam.

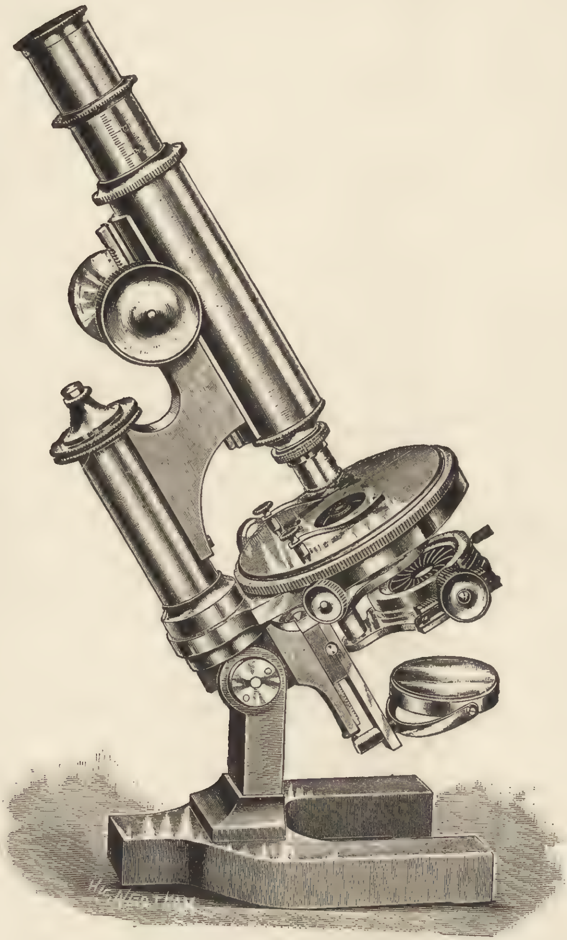
The Typhoid Bacillus. — Whether typhoid fever is caused by a micrococcus or a bacillus is still disputed. The prevailing weight of opinion among the foremost investigators is that it is caused by a short, somewhat thick bacillus with slightly rounded ends, each rod being from $2\,\mu$ to $3\,\mu$ in length and about one-third as thick. The central portion of the cell will not take aniline stains, and gives an appearance closely resembling spores. These spots were long believed to be true spores, and are so described in works of high authority published within a very few years. The most trustworthy authorities now believe that they are not spores, but that the failure of these portions to take the stains is due to the fact that degeneration of the protoplasm is here going on. The typhoid bacilli are found in the mesenteric glands, spleen, liver, occasionally in the kidney, and in the dejecta of typhoid patients, in water that has been contaminated by such dejecta, and in the milk of cows that have drunk of the contaminated water. Cultivation may be made by the methods described in the following chapter, and cover-glass preparations made from the cultures. Cover-glass preparations may also be made directly from the water, milk, or dejecta. A drop is dried on the cover-glass, stained for half an hour with a saturated solution of methyl violet in water, washed, and finished as usual.

The Cholera Bacillus. — The special interest felt at present, and at all times when our country is threatened with the introduction of cholera, warrants a few directions for the study of the cholera bacillus, although the writer has

never had an opportunity to study it personally, and must rely wholly upon the observations of others. The cholera bacillus, or comma bacillus, is a decidedly curved, motile rod with rounded or thickened ends, about one-half or one-third as long, but about twice as thick, as the *Bacillus tuberculosis.* These rods sometimes join end to end, and form a true Spirillum; they sometimes take the shape of the letter O, and sometimes of S. They are found in the dejecta of cholera patients during the early and middle stages of the disease, and in water contaminated from such dejecta.

The method most highly recommended for their direct examination is very simple. A drop of the suspected water, or a bit of the white mucous particles found in the dejecta, is spread in a thin layer on the centre of a glass slip, covered with a very weak, watery solution of methyl violet, and a cover-glass immediately placed over it, any overflow of the staining fluid being taken up with blotting paper. The bacilli take the stain slightly, but do not lose their power of motion, as would, of course, be the case if an ordinary cover-glass preparation were made.

As these bacilli are so minute, the very highest powers of the microscope must be used in the examination. The examination, though difficult, is an eminently satisfactory one on account of the distinctly characteristic form of the bacillus.



LARGE CONTINENTAL MICROSCOPE.
Made by Bausch and Lomb Optical Co., Rochester, N.Y.

FIG. 38.

CHAPTER XIII.

THE STUDY OF BACTERIA: CULTURE METHODS.

Bacteria are such exceedingly minute organisms that with the highest powers of the most perfect modern lenses even the trained eye and brain cannot in many cases definitely distinguish between similar forms. Various methods of cultivation have, therefore, been devised to afford means of studying the habits, effects, and other physiological functions as a means of distinguishing the different species when the forms of the individual bacteria cannot be relied upon. Several species of pathogenic bacteria may easily be mistaken for non-pathogenic varieties, if the appearance under the microscope is the only means employed for their identification. The *Bacillus tuberculosis* and the typhoid bacillus both closely resemble harmless forms.

Another very important end served in the cultivation and isolation of bacteria is the study of the effects produced by the different isolated forms when injected into the bodies of living animals. A simple reference to the researches and discoveries of Pasteur and Koch is all that need be said about the importance of these methods of investigation.

It is the purpose of this chapter to describe in simple form the methods of culture study that have been developed within the last ten or twelve years in some of the leading laboratories of the world, and that are now in common use wherever practical studies of these organisms are being made.

It may be said by way of introduction, that the general method of procedure is to provide proper food material which has been deprived of germs of all other kinds of bacteria, keeping it at such temperatures as are best suited to the variety under consideration. The different species are separated by means which will be described. The food material is deprived of bacterial germs by sterilization.

Sterilization. — Sterilization of a substance means simply heating it to such a temperature that the bacteria in it are killed. No forms can live at a continued temperature of boiling water, but many *germs* resist this temperature for a considerable interval. All germs are killed by heating for one or two hours at a temperature of 160° to 200° C. Apparatus, cotton-wool, everything that cannot be effectually protected from the air, should be so sterilized. It is especially important to fix this fact, and not to use test-tubes and cotton-wool that have not been sterilized for the culture media hereafter described, as contamination is almost sure to follow.

In steam sterilizing, the apparatus and media are submitted to a temperature of 100° C. for half an hour to an hour on the first day. This kills the developed bacteria, but the spores withstand this treatment. After twenty-four hours the spores will have developed, at least partially. They are steamed again for twenty minutes to half an hour, and given another twenty-four hours to develop, when they are again treated in the same way. It has been found that this treatment kills all known germs. Apparatus such as test-tubes and flasks may be plugged with cotton-wool and given this treatment, though it is better to use the higher temperature of dry-air sterilizing, to make sure of killing the germs that fall on the cotton-wool from the air.

The apparatus used in bacteriological work may also be sterilized by washing in various chemical solutions. The substance most commonly used is corrosive sublimate (mercuric chloride). One gramme of the solid dissolved in one litre of water gives the usual 1:1000 solution.

The Sterilizers. — Complicated and costly sterilizers have been devised and used by different investigators, but fortunately very simple and inexpensive forms of apparatus are now in the market for other purposes, which are admirably adapted for bacteriological studies. Both of the articles here described are now in use in prominent laboratories in this country for practical work.

For hot-air sterilizing, the gas stove called the "Cone," manufactured by the Central Oil Gas Stove Co., Boston, does admirably. Glass plates, pipettes, cotton-wool, and any other apparatus, may be sterilized by heating from one to two hours at a temperature of 160° to 200° C. These articles may, of course, be sterilized in the oven of an ordinary cooking-stove. All implements thus sterilized should be carefully protected from the dust until they are cool enough to use.

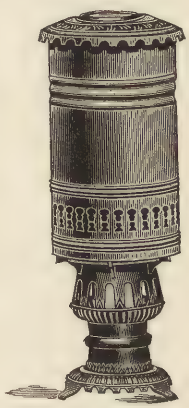


FIG. 39.

For steam sterilizing, the Arnold Steam Sterilizer, which may be purchased for $2.50 of various dealers, or of the manufacturers, Wilmot Castle & Co., Rochester, N.Y., serves the purpose excellently well. It is very simple in construction. It may be used over any source of heat, as a Bunsen burner, oil stove, or an ordinary stove. Water placed in the pan finds its way through small openings into a shallow closed vessel below. This

vessel communicates directly with the sterilizing chamber above. Steam is quickly generated. The sterilizing chamber has an outer jacket placed over it from above. The steam, therefore, accumulates under sufficient pressure to maintain a constant temperature of 100° C. A thermometer mounted in a cork may be inserted through the covers, and, by regulating the flame, any desired temperature below 100° C. may easily be maintained. With the Arnold Sterilizer as the only means of sterilizing, a great deal of thoroughly reliable practical work may be done.

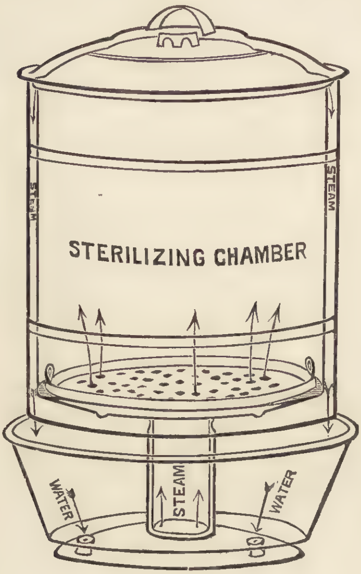


FIG. 40.

Food Materials.—Various substances are used to furnish the bacteria suitable food. Among those most commonly employed are preparations of gelatine and of agar-agar, milk, bouillon, slices of potato, carrot, turnip, and other vegetable substances.

Gelatine Medium.—There are many different formulas for the preparation of this medium. The following is a good one. Half a pound of lean meat is soaked in 500 c.c. of distilled water for twenty-four hours. The meat extract thus obtained is decanted into a flask, and to it is added

Gelatine	50 grammes
Salt	2.5 grammes
Peptone (Merck's)	5 grammes

Allow this mixture to stand a short time for the gelatine to soften, then boil for half an hour. It is now tested with litmus paper for acidity, and dry sodium carbonate added cautiously until it has a faint alkaline reaction on the litmus paper. It is now filtered through a piece of cotton cloth or of flannel, and again through three or four layers of absorbent cotton. As the gelatine hardens too rapidly to allow the medium to be filtered in the open air, the filtering apparatus is placed inside the steam sterilizer, where the filtering is readily accomplished, the sterilizer taking the place of a somewhat expensive hot-filtering apparatus sometimes advised. After the filtering, the medium should be quite clear. One-half of it is now poured into another flask, and made faintly acid by adding acetic acid cautiously until blue litmus paper just turns red. The other half is kept in the alkaline condition. In the subsequent work these two portions are, of course, kept separate, and labelled alkaline and acid.

Test-tubes are now filled about one-third full of the medium, and the mouth of each is tightly plugged with cotton-wool. These test-tubes are placed in the steam sterilizer in wire stands, or simply in empty beakers, to hold them upright, and thoroughly sterilized by heating for half an hour on each of three successive days. They are then ready for immediate use, or they may be kept indefinitely, and used as occasion may require. It is, in fact, a good plan to keep them several days before using, as, if they have not been thoroughly sterilized, a change in appearance will indicate the fact. Paper bags may be drawn over the cotton-wool plugs to protect them from the dust with its contaminating bacteria. If the medium is found to melt in hot weather, the amount of gelatine may be increased to 60 or 75 grammes.

Agar-agar Medium. — Agar-agar is a kind of gelatine made from a sea-weed growing in the Japan Sea. It has recently come into the market for a number of commercial uses, and is now imported into this country by the ton. It is used by some packers of meat to give the meat a richer appearance. It is inexpensive, and may be obtained of dealers in biological supplies. It is used as an ingredient of a culture medium because it will stand a relatively high temperature without liquefying.

The agar-agar medium is prepared by the formula given for gelatine medium, substituting for the 50 grammes of gelatine 5 grammes of agar-agar which has been soaked in salt water for ten or twelve hours, and the salt water drained off.

Agar-agar and ordinary gelatine are sometimes mixed in different proportions, to give a medium combining the qualities of both of these substances.

Test-Tube Cultures. — When it is desired to make a cultivation of bacteria from any source, some of the test-tubes prepared as above are taken. The cotton-wool plug is passed through a flame, ignited, and the flame quickly blown out. The object of this treatment is to destroy any bacteria which may have been deposited on the cotton-wool with the dust that has settled on it from the air. A sterilized pipette with a fine point is touched to the bacteria to be studied, the cotton-wool plug is quickly removed just enough to allow the pipette to be admitted to the tube, and the point of the pipette is touched to or is drawn along the surface of the medium. The pipette is withdrawn, and the cotton-wool plug is replaced. This operation should consume as little time as possible, and every precaution should be taken to prevent dust from the air from entering. The tubes are set aside at the ordinary

temperature, or are kept at some particular temperature, as the conditions of the study may require. The tubes are examined from time to time to watch the development. A turbidity of the gelatine, or translucent or colored spots, after twenty-four to forty-eight hours, show that the bacteria are growing. Cover-glass preparations may at any time be made from the growth.

Cultures at Elevated Temperatures. — Very many investigations, in fact, the larger number, are made at the ordinary temperature of the room. Cultures at temperatures above the ordinary temperatures of the air are necessary in some cases. Disease-producing bacteria, for instance, must be cultivated at about the temperature of the human body. Apparatus for these cultures may be improvised at very little expense. Fill a large tin pan — the larger the better — with water, and heat over a Bunsen burner or oil stove. Provide as a cover a piece of board, through which holes have been bored of a size suited to permit the test-tubes to pass through. Test-tubes with flaring mouths should be used. After the culture tubes are prepared and inoculated, they are passed through the holes in the cover and immersed in the water. A thermometer mounted in a cork is inserted in one of the holes in the cover.

The water is heated with the full flame until the required temperature is reached, then the flame is turned down. With a little experience, it is very easy to maintain a temperature varying not more than two or three degrees in twenty-four hours. The greater the body of water, the easier it is to maintain a constant temperature.

Plate Cultures. — Bell-glasses and plates of window glass about four inches square are required. Ordinary tumblers may be used as bell-glasses. For some purposes shallow dishes, sold in pairs by the dealers under the name of

bacteriological or Petri dishes, are convenient. A pair of these dishes placed under a bell-glass is used in place of the glass plate and tumbler. This apparatus is thoroughly sterilized by dry heat, or by washing with a 1 : 1000 solution of corrosive sublimate.

A tube of gelatine or agar-agar medium is melted by holding it in a beaker of warm water, and is then inoculated with the bacteria to be studied, using the precautions given under tube culture. The tube is thoroughly shaken, the contents poured upon the glass plate, and the bell-glass quickly placed over the plate. The plate is carefully levelled, and the joint between it and the bell-glass is made air-tight by smearing with vaseline. In the course of some hours, if the plate is kept at a suitable temperature, spots caused by the growth of the bacteria will develop. Cover-glass preparations may be made from these colonies, or a cover-glass may be laid over a colony, and, the plate being placed on the stage of the microscope, an examination made.

Method of Isolating Bacteria. — The method commonly employed for the isolation of species is an application of the plate culture. It is usually spoken of as the method of dilution. Several test-tubes of gelatine, or agar-agar medium, are melted at a low temperature by immersing them in warm water. With the usual precautions to prevent contamination from the dust in the air, one of the tubes is inoculated with bacteria. The tube is well shaken, thoroughly to incorporate the bacteria with the medium. A drop or two from this tube is used to inoculate a second tube. This tube is well shaken, and the third tube is inoculated from it, and so on to any required degree of dilution. The contents of these tubes are now poured upon separate sterilized plates, covered with bell-glasses,

and placed aside under suitable conditions of temperature.

After some hours, or in a few days at most, colonies develop, but the plates of the most diluted preparation will, of course, have the fewest. If the dilution was carried far enough, the colonies will not run together, but will be entirely separate. It is accepted that each separate colony thus obtained is the growth from a single individual, or at least from a single chain of individuals of one species. Cover-glass preparations may be made from these colonies, or tubes of various media may be inoculated from them, and what are known as *pure cultures* grown.

Cultures without Oxygen. — Bacteria are called aërobic or anaërobic, according as to whether or not they can live without oxygen. The simplest form of culture without oxygen is made on a gelatine or agar-agar plate. The plate may be prepared by pouring the inoculated gelatine medium upon it, as already described, or the sterile gelatine may be poured upon the plate, and the inoculation made in spots with the point of a pipette charged with the bacteria.

Thin cover-glasses or thin films of mica are now laid over some of the spots, and a ring of melted paraffin is run around each film. Time is given for the growth of the bacteria, and the observation is made whether they grow equally well exposed to the air and under the mica film, where the supply of oxygen is cut off. This culture is a valuable means of distinguishing certain species.

Milk Cultures. — Advantage is taken of the fact that milk is an excellent food for certain species of bacteria. The milk is used as soon as possible after it is drawn from the cow. Test-tubes are filled about one-third full of it, plugged with cotton-wool, and sterilized by heating in the

steam sterilizer for twenty minutes on three successive days. It may then be inoculated in the manner already described.

Bouillon Cultures. — Bouillon, or clear soup, is used in the same way that milk is used. It should be rendered slightly alkaline with sodium carbonate.

Potato Cultures. — Potatoes, carrots, turnips, and other vegetables may be used, either for test-tube cultures or for plate cultures. For test-tube cultures, pieces of boiled potato are cut with a sterilized apple-corer, and these cylinders are cut obliquely from end to end. They are then placed in test-tubes, large end down. This gives a large sloping surface for the cultures. The tubes are plugged with cotton-wool and thoroughly sterilized in the steam sterilizer. They are inoculated in the manner described for tube cultures.

For plate cultures, a boiled potato is peeled with a knife sterilized by passing it back and forth through a flame, slices are cut, placed on glass plates that have been sterilized by dry heat or by washing in the 1:1000 solution of corrosive sublimate, and covered with sterilized bell-glasses. Inoculation with the bacteria under examination is effected by touching a sterilized pipette to the bacteria, and then to the surface of the potato, either in spots or in lines.

Hanging-Drop Cultures. — This is a very convenient form of culture for some investigations. Cells are made by cementing rings of glass, rubber, or metal to microscope slides, as described in Chapter VI. When the cultures are to be made, sterilize the cells by washing in the 1:1000 solution of corrosive sublimate or in absolute alcohol. Smear the top surface of the cell thickly with vaseline. Sterilize a cover-glass in the same way or by passing it through the naked flame, place on its centre a drop of

the sterilized agar-agar or gelatine medium, inoculate it with the bacteria, and invert the cover-glass over the cell, pressing it down gently. The vaseline makes an air-tight joint.

Many of these slides may be prepared at the expense of very little labor. They may be set aside at the required temperature, and studied under the microscope whenever one wishes. The drop of nutrient medium, being attached to the cover-glass, is in a suitable position for examination. If it is desired to keep the preparation moist, a very deep cell may be used, and a drop or two of water that has been sterilized by boiling may be placed in the bottom of the cell.

Another good cell for moist cultures is made by cutting a hole a little smaller than the cover-glass in a piece of thick pasteboard. Soak the pasteboard in boiling water to sterilize it, place it while still wet and hot on the centre of a microscope slide, and immediately place over it a prepared cover-glass with its inoculated hanging drop. To keep up the moisture, wet the pasteboard from time to time in boiled water. If these cultures are to be prolonged for some time, keep the slides under a bell-glass with a dish of water.

A hanging drop of sterilized water, inoculated with the bacteria, affords a most excellent means of studying the movements of the motile forms.

Practical Applications of these Methods. — It is evident from what has previously been said that material for study may be obtained from any putrefying or decaying animal or vegetable matter. In making practical studies of bacteria from any source, the bacteria are first isolated in the manner above described. Inoculations of the pure cultures are then made in test-tubes of alkaline and of acid gelatine medium; of alkaline and acid agar-agar medium; in

test-tubes of milk, bouillon, and potato, both at the ordinary temperature and at different elevated temperatures; plate cultures are made with the different media; cultures are made to determine whether the bacteria are aërobic or anaërobic; and hanging-drop cultures are started.

Observation is made as to whether the bacteria thrive in the different media. The growth is usually easily seen with the naked eye. The colonies are distinguished by the changed appearance of the culture medium, a white, gray, translucent, pearly, or colored spot indicating each growth. Many bacteria in growing liquefy the gelatine medium. The extent to which they do this, and the manner in which they do it, whether in narrow lines extending straight downwards or in diffusely spreading masses, are carefully observed. At any stage of the progress, cover-glass preparations may be made and studied under the microscope. In careful investigations several sets of cultures are made, and the preparations of the same kind are compared with one another to guard against error arising from accidental contamination from the air.

A record of the results should give the source whence the bacteria were taken; the shape and size as observed and measured in cover-glass preparations; the motility as observed in hanging-drop cultures; the temperatures at which the various test-tube and plate cultures thrive best; a description of the appearance of the colonies on the plate cultures; the effect in liquefying the gelatine medium, both the alkaline and the acid; the effect on milk, whether it is coagulated or not, how long it takes to coagulate it, whether the milk is changed in color, and whether it has an acid or an alkaline reaction at the close of the investigation; the effect on bouillon, whether it changes in color or becomes turbid, the character of the

precipitate, if one is formed, whether a skin is formed on the surface; whether the organism can grow without oxygen or not, as observed under the film of mica.

The results of these observations are now to be compared with the observations recorded by other workers, so far as their results can be obtained. It is to be remembered that bacteriology is in its very infancy, and that it is not yet possible to obtain perfectly reliable descriptions of results for a very large number of bacteria. The descriptions given by different workers often differ in important particulars, even those of the very highest authorities. Bacteriology has, however, made sufficient progress to be firmly established as a science. The newness and undeveloped condition of the science make it a peculiarly attractive field for work, but the field is so vast that it hardly seems proper to give in a book of this character more than these general descriptions of the methods employed.

CHAPTER XIV.

PHOTO-MICROGRAPHY.

THE necessity for true representations of objects as seen under the microscope, and the tediousness and difficulty of making accurate free-hand or camera lucida drawings, have naturally called the attention of microscopists to the photographer's camera as a means of securing, with a small expenditure of time and labor, multiplied pictures of microscopic objects. The rapid advances made in the art of photography in very recent years have rendered the accomplishment of this, to a certain extent, within the reach of every microscopist. For certain classes of objects, and for the purpose of fixing the details of structure in the mind of the student, the free-hand or camera lucida drawing must be made, but for many classes of objects, and when it is desired to have numerous reproductions, the photo-micrograph is largely superseding the laborious processes of the draughtsman.

Complicated and costly apparatus has been devised for the accomplishment of special purposes in photo-micrography. Such apparatus is a necessity if the highest power objectives are to be used, as in photographing bacteria and the more delicate diatoms. Special apochromatic objectives, apparatus for using the sunlight, the calcium light, or the electric light, and special orthochromatic dry plates must be used to secure the best results with these classes of objects. Very few private workers would feel warranted in purchasing such outfits. Much may be

done with very simple and inexpensive apparatus. We shall deal here with simple apparatus and with low powers of the microscope, such powers as, in fact, are most serviceable in the general use of the microscope in histological investigations, leaving to specialists the description of special apparatus and special processes. The present chapter proposes to give in detail to those wholly unacquainted with photography, all of the processes from the setting up of the apparatus and the exposure of the plate to the mounting of the finished photo-micrograph. The experienced photographer will prefer processes and solutions with which he is familiar, and numerous modifications may be made of the methods, but processes known to produce good results are here given.

Apparatus. — Any microscope with a tube large in diameter and with jointed pillar, and an ordinary amateur photographic camera, omitting the lens, are all that is required. Such photographic outfits may be purchased at varying prices, but an outfit costing ten or twelve dollars, allowing the exposure of a five by eight inch plate, is advised.

Arrangement of Apparatus. — The eye-piece, together with the draw-tube, is removed from the microscope; the mirror and sub-stage fitting are removed or swung to one side. The body of the tube is inclined at right angles to the supporting pillar. The camera is used without its lens, the microscopic objective being the only lens used. A board four feet long by ten inches wide, placed on any firm table, is used as a support for both camera and microscope, — an arrangement which gives great steadiness. At one end of the board, a rigid, upright support is placed, to which the camera is firmly attached, at such a height that the tube in which the photographic lens is usually inserted

is exactly opposite the end of the inclined tube of the microscope when it is placed in position on the board. The tube of the camera should be of the same size as the tube of the microscope, or larger. The junction of the two tubes must be made light tight. This may be accomplished by winding a black cloth around the tubes, or an adapting tube of tin or brass, painted dead black on the inside, may be used (see Frontispiece). The light to be used is an ordinary kerosene lamp, properly placed to bring the flame in the optical axis of the microscope, no mirror being used, and adjusted to give a clear circle of light on the ground glass of the camera. One of the attractions of photo-micrography is the fact that the exposure can be made in the evening by lamplight.

This simple arrangement is advised for beginners. When some experience has been obtained, a sub-stage condenser may be used; it will be required for the higher powers of objectives. The eye-piece may also be used; the only changes in the position of the microscope and camera will be those required to adjust the focussing. The time of exposure of the dry plate will, however, vary with each different arrangement of the apparatus.

When the apparatus has been arranged as described, light the kerosene lamp and put out all other lights in the room. See that the circle of light on the ground glass is perfect. Place a mounted microscopic object on the stage of the microscope, and fasten it in position with the spring clips. Adjust the objective to about its working distance, and move the ground glass of the camera until the image of the object is seen on it of such size as desired. The image is much more distinctly seen if the ground glass is well shaded from the light. By changing the position of the ground glass and regulating the focus-

sing of the objective to correspond, the object may be projected on to the ground glass variously magnified from a very few diameters to the full capacity of the ground glass.

When a circle of suitable size has been obtained, focus sharply, first with the coarse adjustment, then with the micrometer screw. In the final adjustment of the focus, the image on the ground glass should be viewed with a hand magnifying-glass. It is of advantage, too, to render the ground glass more transparent by cementing with Canada balsam one of the large rectangular pieces of thin cover-glass used in serial mounts to the ground surface. Smearing the ground surface of the glass with glycerine is another means of accomplishing the same end. The image can then be much more sharply seen. Secure the sharpest image possible.

It is now to be remembered that light rays are focussed a little nearer the lens than the chemical rays. Opticians furnish objectives specially corrected for the chemical rays. If an ordinary objective is used, the ground glass is started back very slightly after the sharp image has been obtained; or, what will effect the same result and is usually easier of accomplishment, the objective is slightly moved towards the object by means of the fine adjustment. All is now ready for the dry plate.

Dry Plates. — Any of the reliable dry plates on the market may be used. The beginner is advised to take some one of the popular brands, as the Standard, Harvard, Eagle, or Stanley, and follow the advice of the maker in developing, using the formula for developer furnished with the plates, continuing to use the same brand of plates and the same developer until a considerable degree of skill in their management has been acquired. After this, other plates and other developers may be used, if it seems desir-

able. The beginner should not allow himself to be confused by the many different brands of dry plates and by the multitude of developers; nor should he experiment in different kinds until he has gained sufficient experience to qualify him to judge of the merits of the various kinds. After a time he may make use of orthochromatic plates, which are much superior to the ordinary plate for some classes of objects.

Slow plates are better for the beginner than rapid ones, as slight differences of exposure do not cause so great a change in the plate, and, the development being less rapid, the operator has more time to observe the changes. The Carbutt "B" plate gives excellent results. Plates $3\frac{1}{4}$ by $4\frac{1}{4}$ inches in size are well adapted for practice work. They give a picture of very suitable size for many objects, and are so inexpensive that one does not hesitate to discard a negative that is not perfectly satisfactory and make another trial. The small plate is placed in a *kit*, and the kit in the plate-holder. Dry plates must, of course, be handled in a dark room.

Exposure. — The plate-holder containing the dry plate having been placed in position on the camera, a piece of cardboard covered with black velveteen or other soft cloth is carefully inserted between the object and the objective in such manner as to shut off the light entirely from the objective; it serves the purpose of the lens-cap in ordinary photography. With the high-power objectives the light must be cut off from below the stage, the object and objective being shaded from diffused light by means of a black cloth, or some mechanical means of cutting off the light must be inserted in the camera or the tube of the microscope. The slide of the plate-holder is now withdrawn, and we are ready for the exposure.

No fixed rules can be laid down for the time of exposure. Experience in general photography is, of course, an advantage here, but it is by no means a prerequisite. One without experience is advised to make practice exposures at first. The time will vary with different objectives and with the different objects photographed. The average time for the photo-micrographs reproduced in this book was about eighteen seconds. They were photographed through a $\frac{3}{4}$-inch objective, without condenser or eye-piece, and are magnified about thirty diameters; the dry plates were Carbutt "B." Using similar apparatus and a wood section as an object, the beginner is advised to expose four plates, giving them ten, fifteen, eighteen, and twenty seconds, respectively. Develop, and take proofs to judge of the results. Repeat this practice several times, until the length of exposure is approximately learned. The time, labor, and expense of doing this will be well repaid.

Measurement of Magnification. — After the exposure of the plate, remove the object from the stage of the microscope without disturbing any of the adjustments, and put in its place a stage micrometer. With a pair of dividers obtain the length of the image on the ground glass plate of the camera of any number of divisions of the micrometer graduation. Apply the dividers to an accurate rule to find the exact length of the image. Divide this length by the known value of the divisions of the micrometer scale. For example, suppose a stage micrometer with a scale divided to hundredths of a millimeter is used, and the image of 10 of these divisions, as measured by the dividers and rule, is 7.5 mm. Then 7.5 mm., or 750 hundredths of a millimetre, divided by 10 hundredths of a millimetre gives 75 as the amount of the magnification. This method gives a very accurate result.

If a stage micrometer is not available, the magnification may be roughly calculated, when the objective is the only lens used, on the geometrical principle of similar triangles. The length of the object and the length of its image are the bases of two similar triangles of which the focal length of the objective is the altitude of one, and the distance of the ground glass from the optical centre of the objective is the altitude of the other. The bases are to each other as the altitudes. The focal length of the objective is known, and the distance of the ground glass from the optical centre of the objective can be measured more or less accurately. We have then the ratio of the altitudes, and this is the same as the ratio of the bases. Suppose, for example, that the objective has a focal length of one-fifth of an inch, and the measured distance of the ground glass from the optical centre is thirty inches. Then $30 \div \frac{1}{5} = 150$, the magnification of the image. The results of this method are only approximate, owing to the difficulty of making an accurate measurement of the distance of the ground glass from the optical centre of the objective.

Development. — The development must, of course, be done in a dark room. Any room is a dark room at night, and it is only necessary to provide a lamp giving ruby or yellow light. The plate is taken from the plate-holder, dusted lightly with a soft brush, laid film side up in a shallow dish, and about four ounces of the developer, previously prepared according to the directions accompanying the dry plates, quickly poured over it with a sweeping motion to insure covering the whole surface of the plate at the same instant. The dish is gently rocked to prevent air bubbles or specks of dust from clinging to the plate and interfering with the development.

If the plate has been properly exposed, the image will begin to appear in from twenty to thirty seconds. The development is to be continued until the image begins to lose the bright yellow appearance, and until the exposed portions of the plate where there is no image are quite black when the plate is examined by transmitted light. The development must not be continued too long, or the plate will appear foggy and flat; on the other hand, the development must not be too short, or there will be a lack of detail and contrast. The directions accompanying the formula for developer for producing more or less detail and greater or less contrast should be followed. The whole development should require two or three minutes. Care should be taken that the temperature of the developer is not far from 68° to 70° F.

Some experience is necessary to produce good results. Written directions more than the above are of little value. Experience alone can teach. The pictures produced by hosts of amateur photographers throughout the country show that good work is within the reach of patient workers. The development of a photo-micrographic negative differs in no respect from the development of an ordinary negative, and the fascination in making reproductions of the minute things of nature on an enlarged scale is not at all inferior to the fascination experienced in reproducing the large things on a reduced scale.

Fixing the Negative. — As soon as the negative is properly developed, it is washed thoroughly in clean water. It is then placed in a shallow dish, film side up, and covered with a solution of the hyposulphite of soda, strength one-half pound to one quart of water. One-fourth ounce of alum may be added to this, if desired, to render the film harder and firmer. The negative is to remain in this fix-

ing solution, with occasional rocking, until at least one minute after the opaque yellowness seen on the back has entirely disappeared. All the operations up to this point must be performed in the dark room. The negative may now be exposed to any light. It is thoroughly washed in running water, if possible; otherwise, place it in a large dish and change the water repeatedly. The washing will occupy from one to two hours, and it is very important that it be thoroughly done, as the hyposulphite of soda will turn the negative yellow and ruin it, if any remains in the film. After washing, the negative is set on edge until it is thoroughly dried, when it is ready for printing.

Printing. — Silver prints are the most satisfactory. The ready sensitized silver paper of the dealers is not very reliable, and it is safer to obtain freshly sensitized paper of a local photographer. This paper will keep good only a few days, and so must be used at once. It must, of course, be kept in the dark. It may be cut to the right size with a sharp knife, placing the paper on a glass plate and using a glass plate as a guide. This may be done by lamplight or in subdued daylight.

Place a negative in a printing-frame, film side towards the back. As it is seldom desirable that the whole exposed portion of the plate should show in the photograph, a piece of yellow paper has a circular or rectangular opening cut from its central portion of the size desired for the picture. This cut-out, as it is called, is laid on the negative, a piece of the sensitized paper placed on it, coated side next to the negative, and the back of the printing frame placed in position. The negative is now exposed to the direct sunlight. At short intervals the progress of the printing is examined by turning the spring and lifting one part of the back of the printing-frame. The paper

is so held by the other part of the frame that it will fall back into exactly the same register after the examination. It would not do, of course, to change the position of the paper on the negative. This examination should be made in subdued light. The print should be made somewhat darker than the photograph is desired to be, as it will fade somewhat in the subsequent processes. The print, on being taken from the frame, is placed in a closed box to protect it from the light. When a number of prints have been made, they are ready for toning.

Toning. — The prints are washed in four changes of water, remaining in each about ten minutes. The third washing water should have about an ounce of sodium carbonate thoroughly dissolved in each twenty ounces of water. The prints should be placed in each water face down, one at a time, that they may not cling together.

Two solutions are needed: First, fifteen grains of chloride of gold are dissolved in fifteen ounces of water. Each ounce contains one grain of the chloride of gold, which is sufficient to tone about fifteen three by four inch prints. Second, one ounce of sodium acetate is dissolved in twenty ounces of water.

The toning bath should be prepared at least twelve hours before it is to be used, and is made by adding one ounce of the chloride of gold solution and one ounce of the sodium acetate solution to eight ounces of water. The temperature of the bath when used should be from 75° to 80° F.

Transfer the prints, one by one, face down, from the fourth washing water to the toning solution. Keep them in motion, and be sure that they are not sticking together, or they will tone unevenly. The prints should be examined from time to time by transmitted light. When the

reddish brown color has given place to a rich purple, they are sufficiently toned. They are then transferred to a dish of clean water. All of these operations may be performed in subdued daylight or by lamplight. The prints must now be fixed by dissolving out the unchanged silver salt by means of sodium hyposulphite.

Fixing the Prints. — The fixing solution consists of one ounce of sodium hyposulphite dissolved in one quart of water. It should be freshly prepared, and used but once. Place the prints in the solution, face down, one by one, and keep them in motion for fifteen minutes. This may be done in any light.

Washing the Prints. — The prints are transferred from the fixing solution to water in which a little salt has been dissolved. The salt is used in this first wash water to prevent the prints from blistering. After ten or fifteen minutes the prints are transferred to clean water, which must be repeatedly changed at intervals for several hours. The best way is to place them in a vessel through which water can be kept running in a gentle stream. It is of the highest importance that the washing be thoroughly done, as the prints will turn yellow and fade if even a trace of the sodium hyposulphite is left in them.

Mounting. — After the washing the prints are ready for mounting. They may be mounted at once; but as they have become very tender from the continuous soakings, it is better to allow them to dry first. They are then placed in a dish of water until they are wet enough to be pliable. Then they are laid, face down, in a pile on a sheet of glass. With a small brush apply a thin coat of ordinary flour paste, or of a paste made in the same way from starch, to the back of the upper print. Place the print on a suitable card, and over it a piece of blotting paper. With the hand,

or a small roller covered with velveteen, press firmly on the blotting paper, rubbing from the centre to the outside, until the print is in close contact with the card at all points. Place the photograph between blotters and under a weight until dry, or, if a burnisher is available, run it through the burnisher while still damp. It only remains to attach a suitable label, and the photo-micrograph is finished.

Omega Prints. — A special sensitized paper, known as Omega paper, may be obtained of the dealers. It keeps fresh longer than the ready sensitized silver paper, and may be worked with less trouble. For these reasons, it is a favorite paper with many amateur photographers. The print closely resembles a silver print. It is printed upon in the same way as silver paper, but is toned and fixed in a single solution, which may be purchased ready prepared with directions for use. The prints must be thoroughly washed after toning. It is worthy of recommendation.

Bromide Prints. — Another special paper is known as bromide paper. It is very sensitive, and may be printed upon by lamplight. An exposure, varying with the density of the negative from ten to twenty seconds, to the light of an ordinary kerosene lamp is sufficient. The image must be developed and fixed in the same way as the image on a dry plate, using ferrous-oxalate developer. Explicit directions for using accompany each package of the paper. The prints are in black and white, and are very durable. For some classes of photo-micrographs they are superior to silver prints.

Lantern Slides. — Lantern slides may be made by contact from the negatives of photo-micrographs in the same way as from ordinary negatives. The process is precisely the same as the process of making a bromide print, and is very

simple. Special, slow dry plates, or positive plates, are made for the purpose. In the dark room place one of these over a negative in the printing-frame so that the films are in contact. The exposure is made by lamplight, and must be learned by practice. It varies from five to thirty seconds, according to the density of the negative. The lantern slide is developed in the manner described for bromide prints with ferrous-oxalate developer. The formula for this developer accompanies the plates, and may also be obtained from the catalogues of various dealers in photographic supplies. After development the lantern slide is washed free of the developer and fixed in sodium hyposulphite. The sodium hyposulphite must be thoroughly washed out, for the reasons which have already been given in connection with the other kinds of plates. When the plate is dry, it is covered with a piece of thin, clear glass, an ornamental mat being placed between to cut out the peripheral portions, and the edges of the two pieces of glass are bound securely together by means of strips of black gummed paper.

NOTE.

THE reproductions of photo-micrographs shown in the following plates were made by the half-tone process, a process that gives a decided dark tint to the white portions of a photograph. A good photo-micrograph should show the structure of the object clearly, and at the same time have an undarkened background. The photo-micrographs that are reproduced here have clear and brilliant whites; the dark background of the reproductions is due to the process used.

These photo-micrographs are introduced to give those who are not familiar with the work some idea of the results that may be obtained in low-power photo-micrography. Descriptions of the plates will be found in the List of Illustrations, pages xii–xiv.

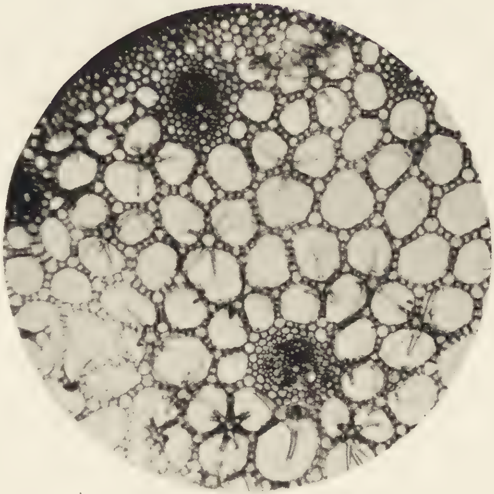
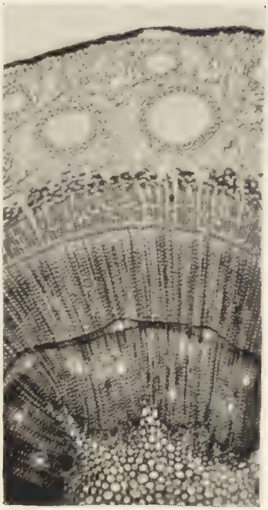


PLATE I.

PLATE II.



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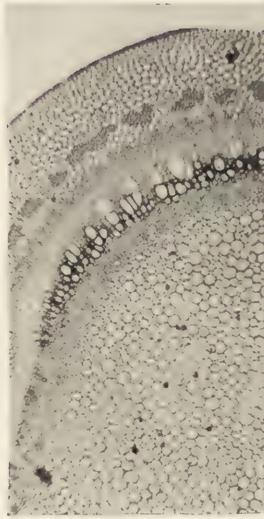

PLATE IV.

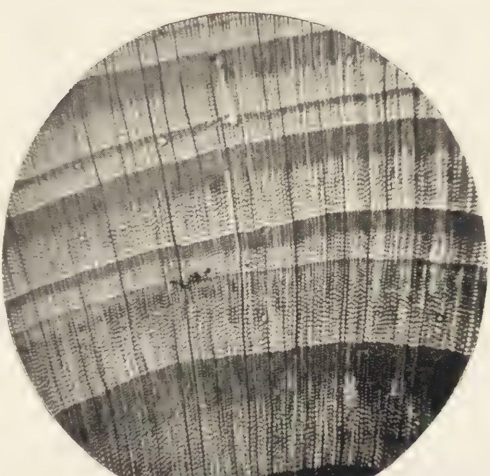

PLATE V.

PLATE VI.

PLATE VII.

PLATE VIII.

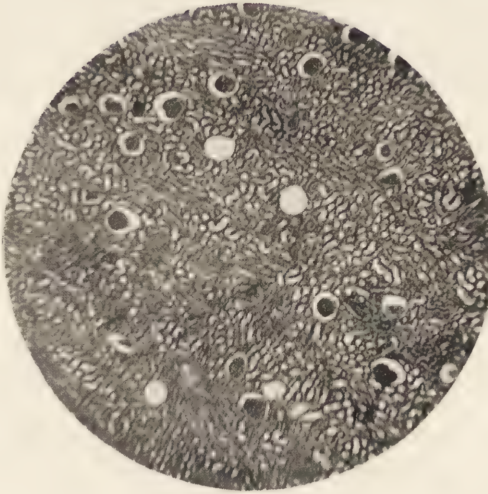

PLATE IX.

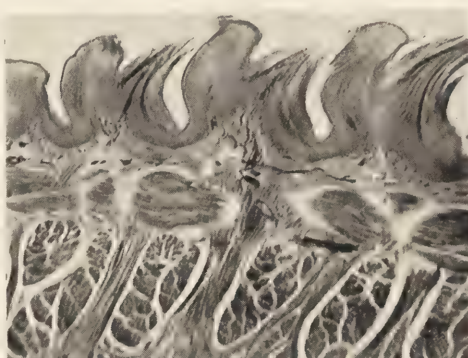

PLATE X.

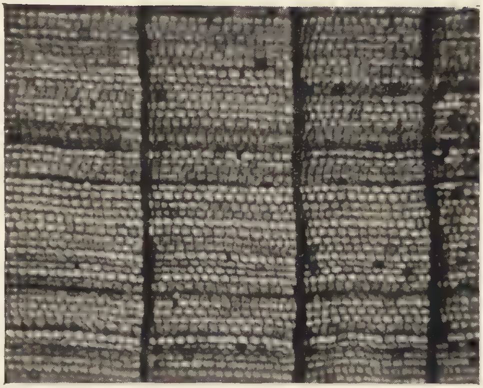

PLATE XI.

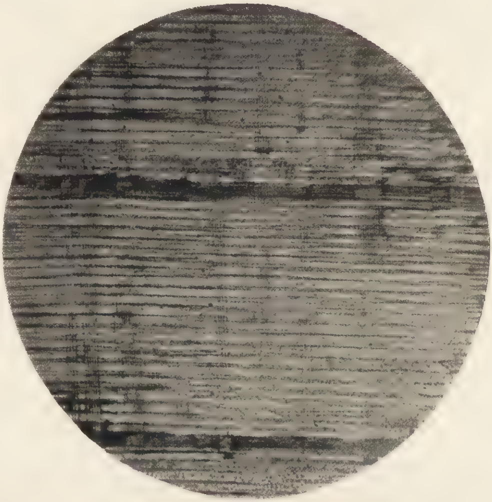

PLATE XII.

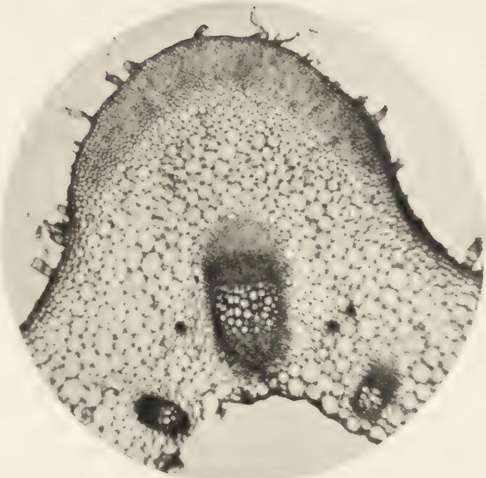

PLATE XIII.

PLATE XIV.

PLATE XVII.

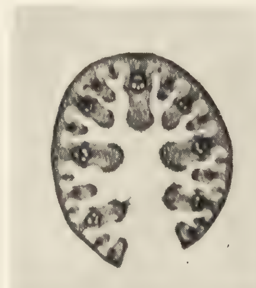

PLATE XVI.

PLATE XV.

APPENDIX.

1. Grenacher's Borax Carmine.

Water	50 c.c.
Borax	2 grammes
Carmine	1 to $1\frac{1}{2}$ grammes

Heat until the carmine dissolves. Add an equal volume of 70 per cent alcohol, and let it stand 24 hours. Filter.

Objects to be soaked in 35 per cent alcohol before staining and washed in same after staining. Excess of stain removed by acid alcohol (alcohol, 35 per cent, 100 c.c.; hydrochloric acid, 4 or 5 drops). A good fluid for staining objects in mass.

2. Grenacher's Alum Carmine.

Water	50 c.c.
Alum (ammonia or potash) . .	1 to 3 grammes
Carmine	$\frac{1}{2}$ gramme

Boil 15 to 20 minutes, cool, and filter. Add a drop or two of carbolic acid to preserve it.

Objects soaked in water before staining, and washed in same after staining. Excess of stain removed by water.

3. Beale's Carmine.

Ammonia	1 c.c.
Water	20 c.c.
Carmine	$\frac{1}{2}$ gramme

When the carmine is dissolved, add 28 c.c. water, and let it stand open to the air, but protected from dust, until the odor of ammonia is faint. Then add 48 c.c. glycerine and 12 c.c. alcohol.

In using, pour a little of the fluid into an open dish with an equal volume of glycerine, and mix thoroughly. The action is slow, requiring many hours. An old fluid is quicker in action than a fresh one.

Objects soaked in water before staining and washed in same after staining, then passed through very dilute hydrochloric acid and again washed in water.

Excess of stain removed by passing object quickly through very dilute ammonia (1 drop ammonia to 5 c.c. water), and then washing thoroughly in water.

4. Gerlach's Ammonia Carmine.

Water	30 c.c.
Carmine	.2 to .4 grammes
Ammonia	A few drops

Mix and, after the most of the carmine has dissolved, filter. Allow it to stand exposed to the air, but protected from dust, until the odor of ammonia is faint. If the carmine separates from the solution, add a small drop of ammonia. For use, add this fluid, drop by drop, to water until the right shade is obtained.

Objects soaked in water before staining, and washed in water after staining. Excess of stain removed by passing quickly through very dilute ammonia.

5. Mayer's Cochineal Tincture.

Alcohol, 70 per cent	10 c.c.
Powdered cochineal	1 gramme

Mix, let it stand some days, and filter. If the action is too intense, dilute with 70 per cent alcohol.

Objects soaked in 70 per cent alcohol before staining, and washed in same after staining.

6. Kleinenberg's Hæmatoxylin.

a. To a saturated solution of calcium chloride in 70 per cent alcohol add an excess of pure alum, and let it stand 24 hours; filter, and add 8 volumes of 70 per cent alcohol. If necessary, filter again.

b. Dissolve crystallized hæmatoxylin in absolute alcohol to saturation. Add this to the first solution until the right intensity of color is obtained. The fluid thus obtained may be diluted at any time by further addition of the first solution. Object soaked in 70 per cent alcohol before staining, and washed in same after staining. Object must contain no acid, or the color will fade.

Excess of stain removed with acidulated alcohol (70 per cent alcohol, 100 c.c.; hydrochloric acid, ½ c.c.). Wash thoroughly again in 70 per cent alcohol to remove acid.

An old solution is better than a fresh one.

7. Grenacher's Hæmatoxylin.

Mix 4 c.c. of a saturated solution of hæmatoxylin crystals in absolute alcohol with 150 c.c. of a saturated solution of ammonia alum in water, and let the mixture stand exposed to the light for a week. Filter, and add 25 c.c. of glycerine and 25 c.c. of methyl alcohol (wood spirit). An old solution is better than a fresh one.

Object placed in water before and after staining.

8. Aniline Stains.

The aniline dyes give more satisfactory results when used in alcoholic solution, though they may be used in watery solution. Make saturated solutions in 95 per cent alcohol of fuchsin, Bismarck brown, iodine green, methyl green, methyl violet, and eosin. Filter, and keep as stock solutions to be diluted with 95 per cent alcohol or water to any desired degree. These solutions do not keep very well. Small quantities, therefore, should be prepared at a time. Excess of stain should be discharged in 95 per cent alcohol. The specimen should be cleared in benzole or oil of cedar. The aniline stains are not very permanent. Objects cannot be stained in them *in toto*, with the exception of Bismarck brown.

9. Fuchsin — Opal Blue. (Double Stain.)

a. Dissolve .1 gramme fuchsin in 44 c.c. 95 per cent alcohol.
b. Dissolve .1 gramme opal blue in 30 c.c. 95 per cent alcohol.
c. For use, mix equal parts of *a* and *b*.

10. Fuchsin — Iodine Green. (Double Stain.)

a. Dissolve fuchsin in 50 per cent alcohol.
b. Dissolve iodine green in 50 per cent alcohol.
c. Add *a* to *b*, drop by drop, until the color is violet.

11. Schällibaum's Collodion-Fixative.

Ether-collodion	1 part
Oil of cloves	3 parts

If this is found not to work well, increase the proportion of collodion. Place a thin coat of this on the centre of a dry slide, place the section on this, and press it flat with a piece of oiled paper. Place the slide in the water-bath until the oil of cloves has evaporated. If these details have been properly attended to, the section will be firmly fastened to the slide. The slide may be passed through successive solutions, and the section will not be loosened. If too much of the fixative is used, there will be a slight tendency to cloudiness. The fixative does not take borax-carmine stain, but does take hæmatoxylin to a small extent.

12. Gelatine Fixative.

Dissolve 5 grammes of gelatine in 30 c.c. of 50 per cent acetic acid. Then add 5 c.c. of 95 per cent alcohol and 1 c.c. of glycerine.

This solution is used to fix diatoms, scales of insects, and other similar objects to the cover-glass or glass slip. Apply the fixative with a brush and let it dry. After the objects are arranged, breathe carefully on it. The moisture of the breath dissolves the gelatine sufficiently to allow the objects to be stuck to it after it has again hardened.

13. Transparent Cements.

The cement used in closing mounts should in every case be transparent. When the mount has been securely closed, and the trans-

parent cement seasoned, colored cement may be applied. Reliable cements may be obtained ready prepared of the dealers in microscopical supplies, and it is perhaps better to obtain them thus. All the cements prepared by Rev. J. D. King, Ph.D., Cottage City, Mass., are very reliable.

Shellac cement may be made as follows: Make a saturated solution of bleached shellac in 95 per cent alcohol. Let this solution stand protected from the dust until, by evaporation of the alcohol, it is reduced to the consistency of a thick syrup. Add a little turpentine to render the cement less brittle. Much of the bleached shellac on the market is made artificially, and is worthless for this purpose. Only real shellac should be used. Shellac dissolves very slowly in alcohol. The bottle should be allowed to stand some days, with occasional shaking.

In applying the cement, keep the brush clean by frequently washing it in 95 per cent alcohol. If the cement becomes too thick, it may be thinned with 95 per cent alcohol. This cement is especially useful for mounts which are to be examined under immersion lenses, as the cedar oil of the immersion fluid does not act upon it, as is the case with most cements. If the mounts have already been ringed with other cements, apply a light varnish of shellac cement.

Berry's hard oil finish, used by painters, is highly recommended as a transparent cement.

14. Colored Cements.

Colored cements may be made by adding to the transparent shellac cement sufficient of a filtered saturated solution in 95 per cent alcohol of fuchsin, iodine green, or opal blue, to give a strong color.

15. Hardening Fluids.

a. For most purposes alcohol, used as described in the preceding pages, is the most satisfactory hardening fluid. As it is somewhat expensive, other fluids may be desired. Other fluids are better than alcohol for some special cases. Whatever fluid is used, the pieces of tissue to be hardened should be small, a large volume of the fluid should be used, and the fluid should be changed frequently, especially during the early part of the process.

b. Müller's fluid is much slower in action than alcohol, the time

varying with different tissues from a few days to eight or ten weeks. The colder the fluid, the better the results. The formula is: —

Distilled water	1 litre
Potassium dichromate, $K_2Cr_2O_7$	25 grammes
Sodium sulphate, Na_2SO_4	10 grammes

c. Erlicki's fluid is recommended for hardening brain tissues, which require about two weeks. The formula is: —

Distilled water	1 litre
Potassium dichromate, $K_2Cr_2O_7$	25 grammes
Copper sulphate, $CuSO_4$	5 grammes

16. Macerating Fluids.

a. Water.

b. Caustic potash, KOH, or caustic soda, NaOH	30 grammes
Water	70 c.c.
c. Potassium hypochlorite, KClO (Eau de Javelle)	8 drops
Water	100 c.c.

d. Alcohol of less than 30 per cent.

e. Schultze's macerating mixture: —

Potassium chlorate, $KClO_3$	1 gramme
Nitric acid, HNO_3	50 c.c.

The last mixture may be used either hot or cold.

17. Mounting Media.

a. Canada balsam, pure or dissolved in benzole, xylol, or chloroform. Best purchased ready prepared.

b. Glycerine.

c. Glycerine-jelly. Best purchased ready prepared. One formula is: —

Best gelatine	1 part
Water	3 parts
Glycerine	4 parts

Soak the gelatine in the water until it is soft. Add the glycerine, and heat over a water-bath. Then add two or three drops of carbolic acid as a preservative.

d. Gum arabic dissolved in water. For vegetable sections.

e. Gum arabic dissolved in a saturated aqueous solution of potassium acetate to the consistency of a thick syrup. (Hoyer's Medium for vegetable sections stained in aniline dyes.)

f. Gum arabic dissolved in a 10 per cent solution of chloral hydrate in water, and one-tenth the volume of glycerine added. (Hoyer's Mounting Medium for vegetable objects stained in carmine solutions.)

g. Gum arabic dissolved in turpentine, or in equal parts of turpentine and benzole. A subsitute for Canada balsam, but much inferior to it.

h. Farrant's Medium for either animal or vegetable objects.

Gum arabic	2 grammes
Water	4 c.c.
Glycerine	2 c.c.

Dissolve the gum arabic in the water and add the glycerine. Stir, but do not shake, to effect solution. Strain through cloth, if necessary.

18. Solutions for Cleaning Glass Slips and Cover-Glasses.

a. Borax dissolved in water.

b. Washing soda (sodium carbonate, Na_2CO_3) dissolved in water.

c. Caustic potash (potassium hydroxide, KOH) dissolved in water.

d.	Potassium dichromate, $K_2Cr_2O_7$ . . .	10 grammes
	Water	50 c.c.
	Sulphuric acid, H_2SO_4	50 c.c.

19. Acids.

Sulphuric acid, H_2SO_4, hydrochloric acid, HCl, and nitric acid, HNO_3, should be used in full strength when the acid is simply mentioned. Special strengths of these acids have been mentioned for particular purposes. For ordinary use, the common commercial acids are sufficiently pure. If, however, the acids are purchased especially for microscopical work, the chemically pure acids should be obtained.

20. Alcohol of Percentage Strength.

In these pages, alcohol of a particular percentage strength has been required for many purposes. It is not always convenient to stop and compute the strength; the following table is therefore added. The strength of ordinary commercial alcohol is assumed to be 95 per cent. It should be this, but often falls short of it. The table is computed not for mathematical accuracy, but for practical utility. It is sufficiently accurate for all the purposes described in this book.

To Make	Use	
	95 % Alcohol.	Water.
95 % alcohol	100 parts	0 parts
90 % "	95 "	5 "
85 % "	89 "	11 "
82 % "	86 "	14 "
80 % "	84 "	16 "
75 % "	79 "	21 "
70 % "	74 "	26 "
65 % "	68 "	32 "
60 % "	63 "	37 "
50 % "	53 "	47 "
40 % "	42 "	58 "
35 % "	37 "	63 "
30 % "	32 "	68 "

21. Iodine Solutions. (Test for Starch.)

a. Iodine dissolved in water. The solution takes place very slowly.
b. Iodine dissolved in alcohol.
c. Iodine dissolved in a water solution of potassium iodide, KI.

22. Chlorzinc Iodine. (Schultze's Reagent. Test for Cellulose.)

Dissolve in hydrochloric acid all the metallic zinc that will dissolve, and evaporate to the consistency of a thick syrup. Add to this solution all the potassium iodide, KI, that will dissolve. Then add all the iodine that will dissolve.

23. Ammoniacal Cupric Oxide. (Test for Cellulose.)

Dissolve in water all the copper sulphate, $CuSO_4$, that will dissolve. Add to this solution caustic soda, $NaOH$, or caustic potash, KOH, as long as a precipitate is formed. Filter, and wash the precipitate in water that has been boiled. Place this greenish precipitate in a bottle and add just enough strong ammonia, NH_4OH, to dissolve it. To test it, see if fibres of cotton will dissolve in it. Keep in a dark place.

24. Solutions used in Photo-micrography.

a. Developer for Dry Plates (Stanley's).

No. 1.

Water	80 fluid ounces
Sulphuric acid	1 drachm
Pyrogallic acid	1 oz. Troy

No. 2.

Water	80 fluid ounces
Sodium carbonate	6 oz. Troy
Sodium sulphite (crystals)	6 oz. Troy

Mix equal parts of No. 1 and No. 2 for use. The same portion of the developer may be used to develop three or four plates. It gives greater intensity after it has been used once or twice than when fresh. If the exposure is known to have been too long, use old developer; if the exposure has been too short, use fresh developer. One part of fresh developer and three parts of that which has been used are recommended for ordinary use on plates that have been exposed normally. Use the developer at a temperature of 68° to 70° F.

b. Ferrous-oxalate Developer (Carbutt's).

No. 1.

Water	30 ounces
Potassium oxalate	8 ounces
Citric acid	60 grains
Citrate of ammonia solution	2 ounces

The citrate of ammonia solution is prepared as follows: Dissolve one ounce of citric acid in five ounces of water. Place a piece of litmus paper in this solution and add aqua ammonia until the paper loses its red color and is on the point of changing to blue. Add enough water to make the solution up to eight ounces.

No. 2.

Water	32 ounces
Iron sulphate	4 ounces
Sulphuric acid	16 drops

No. 3.

Water	9 parts
Potassium bromide	1 part

Mix for use two ounces of No. 1, one ounce of No. 2, one-half ounce of water, and three to six drops of No. 3. This developer may be used for lantern slides and for bromide prints. Temperature 68° to 70° F.

c. **Fixing Solution.** — For negatives, use one quart of water and one-half pound of sodium hyposulphite. One-fourth ounce of alum may be added to this to harden the film, if desired. The addition of the alum gives rise to a precipitate. Allow this to settle and decant the clear solution for use. The alum may be omitted from the fixing solution, and a separate solution of alum may be used to harden the film after it has been fixed, or the use of alum may be entirely omitted. In most cases, except in very warm weather, the film hardens sufficiently without the treatment with alum.

Use for fixing silver prints one ounce of sodium hyposulphite dissolved in one quart of water. The prints remain in this solution fifteen minutes, and are then washed in running water. It is of the highest importance that the washing be done thoroughly, or the prints will turn yellow and fade.

d. Toning solution for silver prints.

No. 1.

Gold chloride	15 grains
Water	15 fluid ounces

No. 2.

Sodium acetate	1 ounce
Water	20 ounces

The toning bath is to be prepared at least twelve hours before it is used. It consists of one ounce of No. 1, one ounce of No. 2, and eight ounces of water. This quantity is sufficient for about fifteen three-by-four-inch prints. When this number has been toned, add another ounce of each of the solutions No. 1 and No. 2. This toning bath will keep for some time, but is liable to spoil if kept too warm. Too great economy should not be exercised in keeping it. Transfer the prints to clean water.

25. Metric-English Equivalents.

1 gramme	=	15.4	grains
1 ounce avoirdupois	=	28.3	grammes
1 ounce Troy	=	31.1	grammes
1 fluid ounce	=	29.25	c.c.
1 pint	=	16	fluid ounces
1 quart liquid	=	946	c.c.
1 quart dry	=	1101	c.c.

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